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GRASSLAND IN BRITAIN[1].

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GRASSLAND, like any other kind of vegetation, represents a balance between the requirements of certain plants on the one hand, and the conditions of the environment on the other. It is a result of Nature's demand and supply. An examination of the environmental conditions which determine the existence of grassland is therefore necessary if we wish to make it and maintain it, or to improve its production. Experimental work has demonstrated that grassland is capable of being improved as to grazing qualities, but the results so obtained can hardly yet be said to be capable of being safely applied to areas beyond those actually experimented upon. Few can say with confidence that such and such a treatment will succeed here, and another treatment there. In other words, we have no basis for classifying or valuing grasslands. Assuming that our present outlook is too narrow, too economic, it may be that by taking a broader, if more philosophical view, some progress may be made.

Since environment evidently has much to do with the development of any kind of vegetation, we propose to consider grasslands less from the herbage composition and more from the ecological point of view of soil, climate, topography and all the other factors which constitute environment.

Experience gained in recent years in this direction has a bearing on grasslands, which we propose to indicate here. If the botanical aspect receives more attention than the strictly economic, it is because it seems a better course to consider the question broadly and in the belief that economics will ultimately find their place in the scheme.

[1] An outline of this paper was read before Section M (Agriculture) of the British Association, Dundee Meeting, 1912.

I. Physiographic and Ecological Aspects.

The present extent and distribution of grasslands in Britain depend on several sets of conditions, distinct, yet interlaced; these include:

1. The economic requirements of each district as determined by the farm-practice, the relation between crops and stock, neighbourhood to markets, prices obtainable for produce, etc.
2. The extent to which grassland has been allowed to take possession in areas where the original natural vegetation has been destroyed by cultivation, deforesting and other processes.
3. The degree to which grazing by sheep and cattle has enabled grassland to supplant the original vegetation.
4. The extent and distribution of original natural areas of grassland which have escaped the plough.

There is good reason for believing that under virgin conditions little of Britain was grassland, and that this only occurred where other types of vegetation could not take precedence, that is on certain kinds of topography to be discussed later. Under man's operations, extensive tracts of grassland now replace what was formerly woodland, heath, moor and marsh. Such grassland is liable to change since it is only a phase artificially introduced into the history of other types of vegetation. Naturally, or with the aid of man, grazing animals have caused other types of vegetation to give place to grassland, and more recently one sees the former area of grassland relapsing into rough heath, etc., through the reduction of sheep-grazing as a result of economic changes, increase of deer forests and other causes. Finally, no statistics are required to show that much land at one time reclaimed and under rotation has been put under permanent grass, or in districts where ploughing is still carried on the land is left longer under grass. It will thus be seen that there is much diversity in the origin and history of grasslands, that some are old, others young, some are natural, others almost artificial.

Our grasslands may therefore be grouped into:

1. Natural. 2. Artificially induced.

Some experience is needed to distinguish these, but this can usually be done through a study of the topography and the herbage, just as a natural woodland can be distinguished from an artificial plantation by the kinds of trees present and the ground-vegetation. The grasslands

believed to be original are confined to certain localities and may further be separated into two groups differing in their origin:

(*a*) The stable types. (*b*) The migratory types[1].

The stable types occur on ground which has been for long periods subject only to slow geological change. Their existence is due to the nature of the physiography, or ('lie of the land'), and of the rocks limiting the growth of tree vegetation and also preventing a state of surface infertility due to leaching and stagnancy. Relatively stable geological features are widespread, but the features of this kind which favour grassland are only found where the rocks have led to certain types of physiography and surface conditions through erosion. Natural stable grasslands are found in Britain on: (1) the chalk downs; (2) exposed hills and ridges of rocks containing an abundance of lime, such as limestones (including some calcareous boulder clays, etc.), and basic igneous rocks such as the isolated hills of dolerite common in Mid Scotland, and so characteristic from their grassy vegetation[2].

The migratory types occur chiefly on areas where surface changes have been recently in operation. They are widely distributed throughout the country, yet are limited to places influenced by the agents of surface change, now or recently; such places are mainly found on alluvial and rain-washed or spring-flushed surfaces along the river and coastal belts, and on the flanks of mountains. These grasslands depend on periodic flushing, flooding and renewal of surface fertility through obviously dynamic agencies. The stable types on the other hand depend on the nature of the underlying rocks and their physiography, and they are static with these factors, changing little under the present climate.

The artificially induced grasslands occupy many types of soil and topography, and differ considerably according to the history of their origin and their treatment. They are chiefly the result of a widespread demand for pasturage and hay in districts where the natural grasslands are too limited for economic requirements. Unlike the natural types they usually show marked variations to all appearances unconnected with the nature of the habitat. Frequently they are very unstable in the composition of the herbage and require constant attention if the higher grazing value is to be maintained.

[1] These have recently been defined at greater length:—Crampton, C. B., "Geological relations of stable and migratory plant formations" (*Scott. Botan. Review*, I. 1912).

[2] Cp. Smith, R., "Botanical Survey of Scotland—Edinburgh District" (*Scott. Geograph. Mag.* 1900); also "Forfar and Fife" (*ibid.* 1904–5); Smith, W. G. and Rankin, W. M., "Vegetation of Yorkshire—Harrogate and Skipton District" (*Geograph. Journ.* 1903), etc

Some types of artificially formed grassland, however, have been so long and so consistently subjected to the same treatment that they have acquired a kind of stability peculiar to them which may be regarded as of secondary origin. On a small scale, lawns and bowling greens and old established college greens are good examples. A less obvious but far more widely extended case of this nature is seen in the old established pastures which are such a feature of the midland counties of England. Our best lawns frequently consist of turf transplanted from stable areas of down-grass or from certain mat types of natural migratory origin. They have to be constantly mown and consistently treated to keep them stable, and usually show change in the herbage in accordance with good or bad treatment. The old pastures, on the other hand, have undoubtedly acquired some degree of secondary stabilisation without such completely artificial methods, and may be regarded as a natural evolution following on the destruction of the original vegetation and a phase of tillage.

We now proceed to consider more closely the conditions in Britain that are favourable for the establishment of grassland, and, on the other hand, those preventing its establishment or leading to its retrogression to other types of vegetation. So far as climate is concerned it is noteworthy that our cold temperate moist summers and open winters favour grassland from sea-level to the highest elevations. Hence grassland cannot be looked upon as altitudinally zonal to climate in this country. Forest is zonal in this respect since the upper tree limit is largely a matter of wind exposure. But the moist cold temperate climate also favours leaching and surface exhaustion in quickly drained places, and leads to soil acidity and accumulation of raw humus or peat, while other edaphic (or soil) conditions unfavourable for grassland are widespread. The increase of atmospheric precipitation (chiefly as rain and mist), both as regards inches per annum and number of days, towards the west and north, and the colder summers especially in the north, carry with them increased surface leaching and accumulation of raw humus and peat. The same increase found toward the north and west is evident as we pass from low to high levels, since few of our mountains rise above the zone of increased winter precipitation. The tendency to superficial leaching and surface infertility or the accumulation of raw humus or peat therefore increases as we pass towards the north and west. As we pass from low to high levels leaching also increases, but the accumulation of humus depends greatly on the nature of the physiography and degree of exposure.

Low summer temperatures, high winds and much accumulation of

raw humus are inimical to most types of forest, but the two first-named are not unfavourable to certain types of grassland. The great physical enemies of grassland in this country are rapid leaching of the surface soil and accumulation of humus. Thus one of the first signs of pasture deterioration in a wet district is the formation of a thick sod of mosses and plant remains so dense that summer rains cannot penetrate to the soil below. Hence surface-rooting species (*Agrostis*, *Anthoxanthum*, *Luzula*, etc.), take possession and deeper rooting species (e.g. white clover) dwindle away. The great competitors with grassland are therefore (*a*) moorland to the north and west and at high altitudes (Figs. 2 and 4), and heath on a rapidly leached topography elsewhere (Figs. 1 and 4); (*b*) forest to the south and east, and in places protected from wind (Figs. 1, 2 and 4); (*c*) marsh, wherever stagnant conditions prevail at low levels (Fig. 3).

The natural conditions which favour grassland in this country are therefore those which prevent:

(1) leaching of the surface in well-drained positions, leading to competition with heath (Figs. 1 and 4),

(2) rapid accumulation of raw humus, and competition with moorland species (Figs. 2 and 4),

(3) stagnancy and souring of the soil in lowlying positions and competition with marsh (Fig. 3),

(4) the growth of forest (Fig. 1).

The factors which prevent leaching on an elevated or well-drained topography may be briefly summarised: (*a*) a finely divided thin residual soil is not leached if it occurs on a soluble or smooth weathering rock basis which constantly supplies mineral nutriment, especially lime; chalk, some limestones and basic igneous rocks (e.g. dolerites) tend to develop suitable soil conditions and topography (Figs. 1 and 2). (*b*) Periodical flushing of sloping surfaces with waters containing alkaline bases in solution, or suspended matter holding these bases, is the natural process corresponding to the artificial top-dressing of basic slag and phosphates. Flushed areas on exposed alpine, coastal or moorland slopes supply these conditions in varying degrees, and on slopes the bright green grassy streaks arising from spring or rainwash flushes are probably known to many as places which sheep frequent (Fig. 2).

The factors which prevent souring of the soil or rapid accumulation of raw humus on areas of flatter topography are:

(*a*) Alternate flooding and rapid drainage which ensure sufficient aeration of the soil on alluvial surfaces (Figs. 3 and 4).

(*b*) A sufficiency of alkaline bases in solution or suspension in places where the drainage is less efficient. Fen, as distinct from moorland bog, is built up by humus accumulation beneath water level, but the later vegetation may become a kind of grassland (Fig. 3).

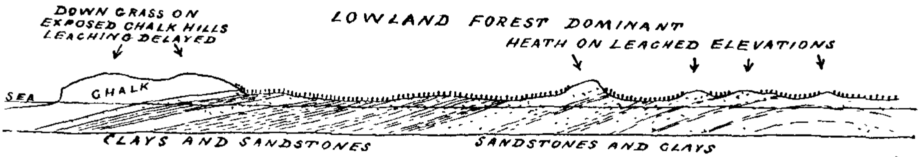

Fig. 1. Diagrammatic horizontal section in South-east Britain to show relations of rocks and physiography to natural vegetation. Lowland Forest dominant. (Verticals exaggerated.)

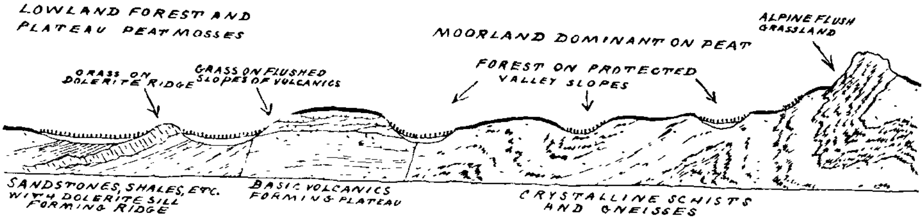

Fig. 2. Diagrammatic horizontal section in North Britain to show relations of rocks and physiography to natural vegetation. Moorland dominant, thick black line represents peat. (Verticals exaggerated.)

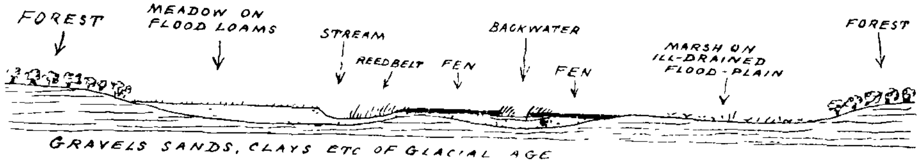

Fig. 3. Diagrammatic horizontal section across a Lowland Flood Plain showing relation of rocks and physiography to natural vegetation. (Verticals exaggerated.)

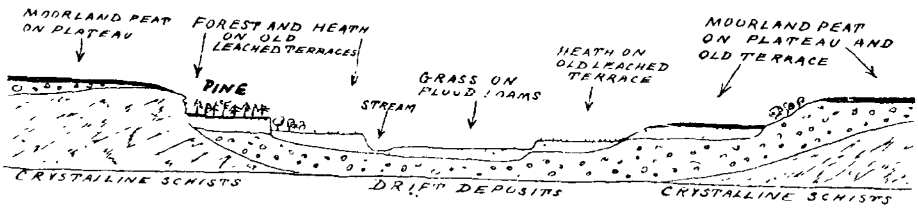

Fig. 4. Diagrammatic horizontal section across a Highland River Basin showing relation of rocks and physiography to natural vegetation. (Verticals exaggerated.)

The factors which prevent the growth of forest are:

(*a*). Great wind exposure as induced by topographic relations to altitude, exposure, and the coast-line. Thus, with increasing altitude there is less protection from wind, but protection varies with the orientation of slope relative to wind exposure, the shape of the surface in depressions or on spurs, and the relations of one mountain mass to another. Coastal slopes are usually exposed, and coastal plains and plateaux are often more wind-swept than mountains.

(*b*) A shallow soil over smooth unfissured rock in exposed positions; this allows no roothold for trees.

(*c*) A water-table too high, or a roothold too unstable on wind-swept alluvial surfaces.

(*d*) The establishment of grassland attracts grazing animals, and these are a most efficient means of suppressing tree seedlings.

The development of grassland in most cases requires a smooth surface not necessarily flat, with a fine-grained soil penetrable in all directions by fine fibrous roots. Except in the case of certain tussock types, mentioned later, there must be a soil and not merely stones and large fragments of rock.

Natural grasslands are therefore restricted (see Figs. 1 to 4) in this country to:

(1) Stable surfaces with a smooth elevated topography and a thin, finely divided soil overlying a rock which supplies alkaline bases, such as the chalk downs, limestone hills, and ridges of basic igneous rocks, and some well-drained surfaces of boulder clay.

(2) Sloping smooth surfaces with a rainwash soil liable to periodical flushing with mineralised waters and rainwash from higher levels.

(3) Periodically flooded and drained alluvial surfaces along rivers.

(4) All surfaces favourable for grass roots in places much frequented by grazing animals.

The influence of the grazing mammals (including rabbits) on the development of grassland is very marked. The grasses belonging to woodland, moorland and heath (e.g. *Brachypodium sylvaticum, Milium effusum, Holcus mollis, Festuca gigantea, Bromus ramosus, Aira flexuosa, Molinia coerulea*, etc.), are rarely pasture grasses, since most of them are hairy, rough, odorous, or otherwise protected against grazing animals. Pasture grasses are those eaten by grazing animals and usually to some extent favoured by the grazing, manuring and mechanical treading of animals. Such grasses generally invade other types of vegetation from disturbed, frequently from flushed areas, and become established through

congregation of grazing animals which prevents the reconstitution of woodland, moorland or heath as the case may be.

Where the disturbance is limited, a neglect by grazing animals is followed by a rapid return to types of vegetation other than grassland. If the causes of disturbance are repeated periodically, the area of grazing usually migrates with the agent, but it may persist and become more extensive if well-stocked with animals. Thus on a moorland or heath flush, the increasing deposits of rainwash deflect the flush-water into new channels and the flush-grassland migrates with it, but constant grazing may maintain the grassland on the parts abandoned by the flush-water. Patches of grassland closely associated with woodland, moorland, or heath should therefore be regarded as migratory plant associations invading these types, not as retrogressive modifications of them, and their existence depends much on the continued operation of animals.

Types of grasslands in Britain may be classified as follows:

I. Turf-forming types. These are usually closely cropped pastures on smooth firm surfaces. They are for the most part perennially green, and are maintained by the vegetative growth of aerial and subterranean leafy shoots, and flower but meagrely. Included here are:

A. Stable types on thin residual soils over rocks undergoing uniform surface solution or superficial complete decay on elevated, exposed, smooth and well-drained surfaces, or on slopes receiving only atmospheric precipitation. These are the down-grass types favoured by a certain degree of summer drought. They consist chiefly of wiry grasses of the sheep fescue (*Festuca ovina* agg.) type along with scattered large-rooted, procumbent, acauline, rosette plants or trailing small-leaved herbs. Mosses are scarce or absent; snails, worms, ants and other invertebrates are abundant. Typical localities are chalk downs, dolerite knolls or ridges, certain maritime slopes and deserted sand-dunes, etc.

B. Migratory types on surfaces of thin rainwash on parts of slopes periodically flushed by occasional springs or the surface run-off from higher levels. These are the mat-pasture types consisting chiefly of flat and short-leaved grasses of forms of *Agrostis, Anthoxanthum, Triodia, Cynosurus,* and others, also often *Holcus lanatus.* Numerous flat and short-leaved sedges of the *Carex panicea* or *flava* type also occur. Mosses like *Hylocomium squarrosum,* or *Hypnum molluscum,* are abundant. Ground invertebrate fauna less abundant than in the down-types, slugs generally replace snails, dipterous larvae generally abundant, ants

usually absent. Much of the vegetation is often glaucous in tint and unwettable. Rosette-leaved plants often have tall flowering-shoots. These mat-types are seen in some alpine grasslands, moorland flushes, and some types of alluvial surfaces near streams.

The *Glyceria maritima* salt-pastures should perhaps be placed with the turf-forming pastures. Along with *Plantago maritima* and other plants, a turf is formed resistent to erosion, but all its members are halophytes and many are succulents.

II. Meadow types. These are characterised by the dominance of taller herbage including grasses of tufted and creeping habit. The soils are generally porous alluvial loams with a high water-table and usually subject to periodical flooding. The meadows require greater summer warmth, hence are found in the lowlands or in sheltered places as riverside and estuarine meadows. This would be an extensive group of grasslands if these soils and topographies were not so much used as arable land. Meadows or "Wiesen" occur at high elevations in Alpine countries with a permanent snow-line, but with warmer continental summers; under these conditions the spring flushing of slopes is widespread and gentle[1], and the effects of leaching and humus accumulation are less pronounced than in Britain. On a small scale we have in Britain similar grasslands in protected sunny places on many of our higher mountains.

The meadow types differ markedly in their summer and winter aspects, and depend upon some degree of summer warmth for their growth, and upon floods, snow, or cattle for the removal of hay or old herbage. Tall mesophytic herbs of the *Spiraea ulmaria* and *Angelica* types are frequent, and many of the plants are highly aromatic. *Lepidoptera* often abundant. The grasses of the lowland types in this country are apparently represented by species of broad-leaved Fescues, *Poa trivialis, Dactylis, Phleum, Alopecurus,* etc. Maritime types have *Hordeum, Phleum, Lolium, Triticum,* etc., and Alpine types have Alpine forms of *Festuca, Aira, Phleum, Poa,* etc.

III. Tussock types are composed of coarse, hard or wiry grasses which tend to accumulate soil by means of stools or tussocks of dead shoots, and usually carry their dead leafage throughout the resting season. Grazing animals generally avoid these grasses but, as in the case of *Nardus stricta,* may take the young spring shoots. They occasionally

[1] The turbid water from melting glaciers ("glacier-milk") is highly valued in the Swiss Alps, and is used to irrigate the high pastures (Wiesen) in many parts.

form a closed association but on rough stony ground the tussocks are more or less scattered. The tussock types are widely represented in various parts of the world, as on salt-steppes, wind-blown steppes, and under conditions of perennial cold or wind. They are capable of surviving intensely unfavourable conditions of surface and exposure, especially scour by wind and water. Their evolution seems to depend more on the physical environment than the biotic, and they do not attract animals, nor are they dependent on continued grazing.

Although in other countries the tussock types may perhaps form stable grassland, in Britain they appear to be naturally limited to unstable habitats unfavourable to other types of vegetation. Thus they are frequently the first grasses to occupy loosely the waste heaps of mines or quarries in exposed places. On a larger scale *Nardus* grassland and sometimes an *Agrostis* type occupy areas subject to much scour by streams, also on sterile soils subject to wind-action, and on peat surfaces where snow lies long in winter. The extent to which these types have spread naturally needs further investigation, but it is a prevalent belief that "white-grass" (*Nardus*) takes possession of many places where heather has failed to recover quickly after burning. The natural centre of the *Nardus* type is in scoured moorland flushes but it has probably invaded wide tracts of moorland which have undergone degeneration from artificial interference, and its replacement by more acceptable types of grassland is an important problem in many sheep-grazings. The *Agrostis* type is liable to dominate degenerated artificially formed pastures, especially in bleak places where sheep are grazed without cattle; the introduction of the hardier cattle is a recognised means of improvement of the type, and should probably precede top-dressings of basic slag or phosphates.

The bristle-leaved Fescues also assume the tussock habit in exposed coastal regions.

IV. The Stooled Meadow types. These might be considered as exaggerated forms of the tussock types; they assume the stooled habit rather as an adaptation to frequent gentle flooding and silting, or to standing water, than to scour by wind and water. They accumulate silt more rapidly and raise themselves above the flood-level and frequently form a belt round lakes and along still streams between the reed-belt and aquatic marsh plants, and the more typical meadow types. The principal grass-types are *Aira caespitosa* and *Molinia coerulea* in its large stooled tall-growing Fenland form. Tall rushes and stooled species of Sedge (e.g. *Carex paniculata*) are often present.

V. Lair-grasslands and the Camp-follower types. There still remains a somewhat heterogeneous group of grasslands which occurs where from the physiographic and soil-factors one would expect to find other types of vegetation, but grasses take possession because grazing animals either willingly or perforce congregate there. The natural congregation of grazing mammals, say for shelter, tends strongly to favour grassland, and the enclosure of sheep or cattle within walls or fences has the same effect. In this country rabbits lead to the formation of types of grassland somewhat resembling down grass on all well-drained surfaces they frequent. Much of the grassland of fixed dunes is evolved through their agency, though the same effect may follow sheep grazing.

Wind-breaks in woods and other breaks resulting from death of trees or damage by animals, favour the invasion of grasslands of different types; these depend to a large extent on the type of grassland in the vicinity and on the kind of grazing animal. Sheep and rabbits tend to produce mat or down-like types according to the nature of the surface. Cattle induce types ranging from mat to meadow according to soil and moisture. If the animals fail to produce a suitable type of grassland, they desert the place and other types of vegetation come in.

When we turn to the large stretches of grassland of artificial origin in this country, the effect of fully stocking a reclaimed surface with sheep and cattle is more apparent. Wherever the ground has been cleared and tilled in the lowlands, especially in the south and east, a full stocking with animals would probably be sufficient to produce some kind of grassland even without sowing of grasses. The forest, which was here the chief competitor of grasslands, has been eliminated by a course of deforesting, reclamation and prolonged agricultural operations over wide areas, so that many square miles are now occupied by old grassland, which so long as appropriate treatment is followed remains relatively stable. On the moorland surfaces in the north and west the unfavourable peaty conditions generally lead to rapid deterioration of grasslands of artificial origin. Where the moorland still holds a peaty inheritance, or the topography or porous nature of the surface leads to rapid leaching, the artificial formation of grasslands is difficult and necessitates such operations as removal of peat, ploughing, liming, etc. It is in such places that stocking with sheep may still fail to prevent deterioration of the herbage and the reappearance of heath or moor, or the barren tussock types of *Nardus* or *Agrostis*.

The origin of the grasslands of reclaimed ground is an intricate

problem, but we consider that the more permanent pastures, where the influence of the original sowing may usually be neglected, probably closely resemble the natural migratory grasslands of that area; these formerly were restricted to certain areas where migratory factors excluded other types of vegetation.

If a patch of ground is cleared in the midst of forest or other natural vegetation of a stable kind, it is rapidly invaded by plants from two quarters: (1) plants from migratory associations in the vicinity, and (2) plants from the surrounding stable associations. Provided no further interference takes place the plants of the stable associations may gradually oust all other competitors and the stable formation is reconstituted. This may be seen in small enclosures in hilly districts or in woodland clearings. If, instead of a small patch of ground being cleared of its original vegetation, a wide district, such as the Midlands, gradually undergoes a transformation of this kind, the immigration of plants from the original stable associations is checked and eventually eliminated. The ground which formerly supported stable associations if left to itself can only become clothed by types of vegetation constructed from such plants as were the least easily exterminated or the most rapid in migration. These plants are those of migratory formations, since these habitats are generally governed even in the most fertile districts by geological factors which have hindered their complete reclamation by man.

In accordance with this we find that limited artificial clearings in our moorland districts are soon recaptured by the moorland associations, whereas those in widely cultivated districts are often for a time occupied by a motley assemblage of weeds, incapable of reconstructing any associations comparable with those of the original vegetation.

But certain types of migratory associations depend on the influence of grazing mammals for their maturation and persistence to an even greater extent than on the geological factors which were often an initial cause in their formation. Chief amongst them are types of grassland which largely owe their degree of stability to the constant presence of such animals.

II. The Economic Aspects

In the preceding we have attempted to point out the natural factors which lead to the formation of grasslands, and how in the absence of these factors other types of vegetation supervene.

The economic aspect of grassland has usually been approached from

a very different standpoint. Where exact data are attempted they are usually obtained from the experimental side, or by a compilation of the scattered information known to the shepherd or grazier. The usual routine of the farmer in obtaining grassland is well known. It differs according to the local conditions and the requirements which determine the rotation of crops. A fine surface tilth, a mixture of seeds determined by experience, sometimes by fashion, a smooth well-rolled surface, and a treatment of the crop to obtain the best results, is practised so far as grassland in rotation is concerned. It is, however, with permanent grassland and unenclosed grazings that ecological investigation has made most progress. Many valuable papers have been written on the reclamation of moorland, heath and marsh, and on the improvement of hill pastures, chiefly records of practical experiments successful or otherwise[1]. Little, however, has been said as to the causes which lead to the appearance of grassland on the one hand and to its degeneration on the other.

What we have to say at present is chiefly deduced from a consideration of the natural factors already indicated. Where these factors are naturally in operation and stable in character as on chalk downs, no artificial interference is needed to procure good sheep-grazing, and no other treatment than judicious stocking should be attempted until well-established results based on experiment are obtained. In the case of natural migratory grasslands the case is otherwise since the natural factors in these cases are dynamic in operation and such as can to some extent be controlled by man. Such control has, in fact, long been practised in the formation of water-meadows, in dyking marshland and in the warping of estuarine flats. But there is always a danger of meddling unnecessarily with natural migratory grasslands, and it is necessary to consider carefully in each case the physiography or "lie of the land," the nature of the waters and their suspended materials, and the effect of tampering with the drainage. While the natural flushing of peaty moorland by water often leads to the appearance of patches of grassland (i.e. moorland flushes), the conduction of such waters on to the grasslands of the stream alluvia will in most cases promptly lead to marked deterioration in the pasture. This is recognised in many upland districts, and the invasion of the peaty water over the alluvial grasslands is prevented by a system of drains which intercept the moor water and

[1] Many papers of this kind will be found in the *Transactions of the Highland and Agricultural Society of Scotland*; they refer to all parts of Scotland and date back to 1799. See also *Journal of the Roy. Agric. Society*.

conduct it direct to the stream. Peaty waters invading areas of alluvial sandy loams frequently lead to the formation of pan with stagnation of the surface and a reversion to marsh or moor. Again, the underdraining of sandy loams only accelerates the leaching natural to them, and thus favours heath unless frequent surface manuring with lime or slag is carried on. The periodic flooding of sandy loam alluvia is a natural means of renewing surface fertility, and it is doubtful whether in some cases it is not wiser to allow them to be flooded than to enclose them against flood-waters.

The grazing and manuring of sheep and cattle on moorlands often do much to establish grassland where migratory geological factors are in operation, but it is probable that no amount of overstocking will be effective if leaching has reduced surface fertility and induced acidity. Lime is the saving factor in most of these cases, while basic phosphates and potash may cause a complete transformation if they can be used with profit. Many results of such experiments have been published[1]. A top dressing such as basic slag has frequently resulted in great increase of leguminous plants (White Clover, etc.). This effect may be the result of increased available mineral matter in the soil, or improvement due to increased activity of soil micro-organisms in a more alkaline soil. The subsequent improvement of the herbage is also largely due to more intensive grazing; "the bite has been sweetened" (we have seen dressings of salt almost as effective as a bait) and more uniform grazing has favoured some grasses while reducing others which formerly were neglected and tended to monopolise the pasture. In certain cases there is less alteration of the species of plants than might be expected from the improved appearance, the existing species under more intensive grazing assume another form.

Low-lying alluvial clay loams are long in leaching, and so far as grassland is concerned they suffer more from insufficient drainage. This is especially the case where they form the alluvia of moorland rivers rich in derivatives from moorland peat. Such grasslands may be protected from flooding by straightening the rivers and limiting them by raised banks. In all cases where the natural agents which led to the formation of the grassland are excluded, impoverishment follows unless the soil, drainage, and exposure are sufficient to counteract

[1] The numerous published reports are conveniently summarised by Professor W. Somerville ("Influence on the production of mutton of manures applied to pasture," *Journ. Board of Agriculture*, XVII. Supp. 5, 1911); for the Rothamsted results consult *The Book of the Rothamsted Experiments*, by A. D. Hall (London, 1905).

this. Such reclaimed alluvial clay loams frequently include a supply of humus and mineral nutriment which may be rapidly exhausted or properly conserved according to their treatment.

The grasslands of the dyked levels in this country are maintained under artificial conditions by elaborate systems of drainage. So far as can be ascertained much of this ground was originally reed-belt and fen vegetation, with areas of marsh and grassland of the meadow and stooled meadow types in more elevated parts and round the margins fringing the higher lands. Artificial interference in their reclamation has reduced the whole surface to what looks a uniform type of pasture. But recent experiments have shown that fields in the Romney marsh levels to all appearance alike, and which even proved to be almost identical as far as soil and botanical analysis of the herbage are concerned, have quite different values for the fattening of sheep[1]. This is assumed, and probably rightly, to be due to differences in the same species of plants in the different fields. If this be so it proves that botanical analysis so far as the species of plants and numerical proportion of individuals are concerned, is not an exact method for ascertaining the economic value of a grassland.

Field ecologists are well aware that the same species of plant may take on very different growth forms, sometimes easily traced to the habitat they frequent, at other times for reasons not easily perceived. It is also known that plants have medicinal properties differently developed according to the district from which they are derived. With regard to grasses, recent work at Svalof (Sweden) and in Denmark shows that pasture and meadow grasses (e.g. Timothy (*Phleum pratense*), Cocksfoot (*Dactylis glomerata*), Meadow Fescue (*Festuca pratensis*) and Tall Oatgrass (*Arrhenatherum avenaceum*)) include a large number of forms differing very distinctly in height and habit, some remaining constant under cultivation[2]. It is therefore not unlikely that some of these forms of grasses have been evolved under the artificial conditions of highly stocked pastures. The rich pastures of the Midland counties may well owe their stability not merely to isolation from virgin plant associations, but from a gradual natural selection of those forms of grasses and other plants which are most suited to the treatment in vogue, including grazing.

[1] Hall, A. D. and Russell, E. J., "On the causes of the high nutritive value and fertility of the fatting pastures of Romney marsh and other marshes in the S.E. of England" (*Jour. Agric. Sci.* IV. p. 339, 1912).

[2] Witte, H., "On polymorphy of the more important forage grasses"; in Swedish with summary in German (*Sveriges Utsädesförenings Tidskrift*, XXII. Häfte 1 and 2, 1912).

Certain grasses as forms of *Poa*, Cocksfoot, and sometimes Ryegrass (*Lolium*) grow luxuriantly wherever man has been in residence, sometimes in isolated places far into the moorland. They are often referred to in ecological works as a "Lair-flora." Similar plant associations occur in sheep-walks in richly manured places where the sheep lie. Other types of *Poa* associations are met with on ledges of sea-cliffs frequented by gulls in the breeding season, and on inland bogs the vegetation frequently assumes a rank grassy type where gulls nest. All these types may be regarded as camp-followers of man and animals, and it is probable that the extensive green pastures of our more civilised districts are really extended associations of a somewhat similar nature. In these 'culture-types' if we may so call them, different species of grasses are included and form very different associations. These types are largely represented in old established cultivated districts and as smaller patches in the wilder places. Their habitats are apparently very resistant to the effects of leaching of the surface, and to soil exhaustion. In some cases the soil may have such a stock of nitrogenous matter, partially renewed by grazing animals, that the demands of these lair grasses are satisfied. On the other hand in the grasslands usually encountered in the wilder and more inclement regions of our country, the effects of leaching are rapid and marked. These "hill pastures" consist largely of species of *Agrostis* with *Anthoxanthum*, and species of *Festuca*, etc., usually forming a shallow turf and living as it were on the minimum of surface nutrition for pasture grasses. A want of sufficient grazing and an accumulation of dead hay and humus or "sod," a slight deficiency in the nature of the manure supplied by stock, or too long a continuation of leaching easily turns the scale in these types of pasture, so that mosses and heath plants once more settle down and displace the grasses.

No attempt has been made here to discuss specific cases of treatment for improvement of grasslands. In this direction it seems more probable that careful experiment will lead to better conclusions than botanical analysis alone. The plants composing the herbage should of course be ascertained, because they will give some guidance in classification, and by using the names of dominant plants a convenient nomenclature may be established[1]. Experiment, however, will require to be accompanied by other observations than usually appear in the published report. The locality itself needs further consideration with regard to physiography, origin, and history, such as we have attempted to outline here. An

[1] Methods of botanical analysis, see Rothamsted Memoirs; Armstrong, S. F., *Jour. Agric. Sci.* II. Part 3, 1910; Stapledon, R. G., *ibid.* V. Part 2, 1913.

interpretation along these lines of the results observed by application of manures or other treatment seems the more logical method. Thus if a certain treatment is beneficial on porous, "hungry," stream alluvial belts where leaching has led to impoverishment of the herbage, then similar treatment will probably be profitable on other stream alluvia similar in situation and soil composition; but it does not follow that it will benefit grassland on stiff heavy stream alluvia laid down under different conditions. Again, results obtained on the pastures of porous and leached valley or hill slopes cannot be applied to other areas where much peat is present unless experiment has shown that this is the right method. The test of success in experiments probably lies in the effect on grazing stock, and the decision will rest with the grazier.

THE NATURE AND AMOUNT OF THE FLUCTUATIONS IN NITRATE CONTENTS OF ARABLE SOILS.

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THE study of the fluctuations in the amounts of nitrate present in arable soils is important both from the practical and the scientific points of view. The nitrate supply in the soil is very commonly a limiting factor in crop production in Great Britain, so that any process which increases the nitrate supply tends to increase productiveness, and vice versa. From the scientific point of view the interest is even wider. No soil constituent, not even the moisture, shows such great fluctuations as the nitrates, and none is so susceptible to external influences. Further, the nitrate represents the end point in one chain of decompositions and the amount formed over a given period is therefore a measure of the extent to which this particular decomposition has proceeded.

The experiments of Warington, Omelianski[1] and others have proved, as clearly as any negative proposition can be proved, that nitrates are formed only from nitrites and these only from ammonia. The formation of nitrate has been shown in an earlier paper[2] to be the quickest of these stages while the formation of ammonia is the slowest. The proof is that under no natural conditions have we ever found any accumulation of ammonia in the soil. At Rothamsted the amount found is a constant minimum, about 1 or 2 parts per million of soil. Such a negative proof is obviously not rigid, but so far as all the evidence goes this non-accumulation of ammonia is characteristic of soils kept under natural conditions of temperature, moisture and aeration.

[1] *Centr. Bakt. Par.*, Abt. II. 1899, **5**, 473—493: "Ueber die Nitrification des organischen Stickstoffes."

[2] This *Journal*, 1909, **3**, 233.

The amounts of nitrate in the soil at different periods of the year.

Systematic determinations of the nitrate in the soil of arable land at different times of the year bring out the very remarkable fact that the amount of nitrate is commonly at a maximum, not in late summer as is often stated, but in late spring, or early summer. This is shown in Table I where the means of all the results on uncropped land[1] are collected, and in Table II where the results of cropped land are given. The highest amount, or nearly the highest, occurs in May or June, after which there is sometimes a slight increase but sooner or later a fall. What is even more remarkable is the rapid rate of accumulation of nitrate in the spring; the rise from the winter minimum to the early summer maximum is very rapid, and usually much quicker than anything obtained later.

After the mild wet winters of 1911—12 and 1912—13 this rapid accumulation of nitrate did not set in directly the warm weather began; there was a well marked lag. Thus in 1912 there was no increase in the stock of soil nitrate during the month of May but a very marked increase during June. Yet the weather appeared to be very favourable in May as shown by the following data :

	Period of no accumulation of nitrates, April 28—May 25				Period of marked accumulation of nitrates, May 26—June 22			
	1st week, Ap. 28—May 4	2nd week, May 5—11	3rd week, May 12—18	4th week, May 19—25	1st week, May 26—June 1	2nd week, June 2—8	3rd week, June 9—15	4th week, June 16—22
Rainfall, inches	0·40	0·03	0·22	0·32	0·43	1·20	1·02	0·12
Mean temp., °F.	49·0	58·9	54·8	52·2	53·4	54·0	56·1	60·0
"Accumulated heat" (day degrees above 42° F.) ...	61	118	90	71	81	84	99	126
	April 30	May 13		May 22				June 26
Moisture in soil, per cent. (Plot 7[2])	17·5	14·8		15·5				19·3
Nitrate in soil, parts per million (Plot 7[2])[2]	15	18		12				28

[1] Fruit trees were growing on the loams and the clay but the land was kept cultivated and free from any quick growing crop.

[2] Similar data for other plots will be found in Table II. Pouget and Guiraud (*Compt. Rend.* 1909, **148**, 725), working at the School of Agriculture, Maison-Carrée, Algeria, found

Again in 1913 no accumulation of nitrates took place during May in spite of the favourable weather, but a rapid rise set in during the first week in June:—

	Period of no accumulation of nitrates, May 11—31			Period of marked accumulation of nitrates, June 1—July 5				
	May 11—17	May 18—24	May 25—31	June 1—7	June 8—14	June 15—21	June 22—28	June 29—July 5
Rainfall, inches	0·07	0·06	0·31	0·35	0·31	0·34	0·06	0·28
Mean temp., °F.	51·8	52·2	61·5	55·2	55·1	59·2	57·0	58·4
Accumulated heat (day degrees above 42° F.)	69	71	137	92	92	120	105	115
		May 19	May 31		June 9		June 23	July 9
Moisture in soil, per cent. (Agdell, Plot 2)		16·3	14·5		16·0		12·2	13·3
Nitrates in soil, parts per million:—								
Agdell, Plot 2		7	4		11		3	18
,, Plot 1		8	5		15		4	14
Hoos 1·0		5	5		18		4	21

None of these plots received any manure during the season.

In both cases the preceding winter had been mild and wet so that the soil had lain wet for many weeks. In 1909, after a drier and colder winter, the rise set in at an earlier date and was already manifest on some plots early in April, although the weather appeared to be distinctly unfavourable (see Table on opposite page).

The subsequent changes in the amount of nitrate differ accordingly as the land is cropped or not. Where there is no crop the nitrates accumulate to a greater extent than where a crop is growing; but as

that nitrates almost entirely disappeared from the soil in the wet months, Jan.—April, but did not at once begin to accumulate when drier, warmer weather set in. Only little nitrate was found during May, although the mean temperature for the month was 18·3° C.; not till June was the accumulation at all marked. The results obtained were:

	Feb. 12	Feb. 27	April	May 22	June 6	June 13	June 20
N. as nitrate, parts per million	2	1·5	trace	3·7	6·3	7·6	7·3
Mean temperature for the month, °C.		8·8	11·9	18·2	18·5		
Rainfall for the month, mm.		93	76·5	10·8	9·7		

the crop introduces a new factor we shall begin by discussing the case of land either wholly fallow or else only carrying young fruit trees, where the complication due to the crop is reduced to a minimum.

	1st Period, March 7—April 10					2nd Period, April 11—May 8			
	March 7—13	March 14—20	March 21—27	Mar. 28—Apr. 3	April 4—10	April 11—17	April 18—24	Apr. 25—May 1	May 2—8
Rainfall, inches	0·29	0·53	0·65	0·99	0	0·20	0·64	0·83	0
Mean temp., ° F.	35·8	37·1	43·5	43·4	44·8	48·4	49·0	46·9	48·4
Accumulated heat (day degrees above 42° F.	−43	5	26	29	68	60	54	47	60

	April 6		May 7	
	Broadbalk Orchard	Hoos dunged Plot 7[2]	Broadbalk Orchard	Hoos dunged Plot 7[2]
Moisture in soil, °/₀...	15·5	20·0	13·7	18·0
Nitrate in soil, parts per million	4	22	8	21

1. *Land wholly or mainly left fallow. Effect of soil.* Reference to Table I shows that under similar treatment the sand is at all times except winter poorer in nitrates than loams or clays. The highest amount was recorded in May 1909 when 8 parts per million were found in the top 9 inches, or 36 lbs. per acre in the top 18 inches, but more usually only about half these quantities are present, and during the hot dry summer of 1911 the amounts fell to 2·5 parts per million, or 13 lbs. per acre in the top 18 inches.

In winter and early spring the loam contained approximately the same amount of nitrate as the sand, but later on—from May onwards—it contained much more. The maximum amount found at Ridgmont was 19 parts per million in the top 9 inches, or 95 lbs. per acre in the top 18 inches: at Rothamsted still higher quantities (23 parts per million or 115 lbs. per acre) occurred in the hot dry summer of 1911.

The clay contained more nitrate in winter and early spring than the loam but less in summer and autumn. The amount never fell below 4 parts per million, or 20 lbs. per acre in the top 18 inches, nor, on the other hand, did it rise above 14 parts per million, or 60 lbs. per acre; there were no sharp falls and no sharp rises.

Comparing the results for the three types of soil it is evident that the loams are most suitable for the accumulation of nitrate, next comes the clay, while the sand is the least suitable. The sand and the loam

lose their nitrates equally completely in the winter, the amounts running down to 9 lbs. per acre, or 1 part per million. The clay suffers much less loss, the lowest amount found being 20 lbs. per acre or 4 parts per million. Thus the total fluctuation is least in the clays and most in the loams. These relationships are shown in the curves for 1912 in Fig. 1. It will be shown later (p. 49) that organic matter conditions the retention of nitrates during winter just as clay does, the

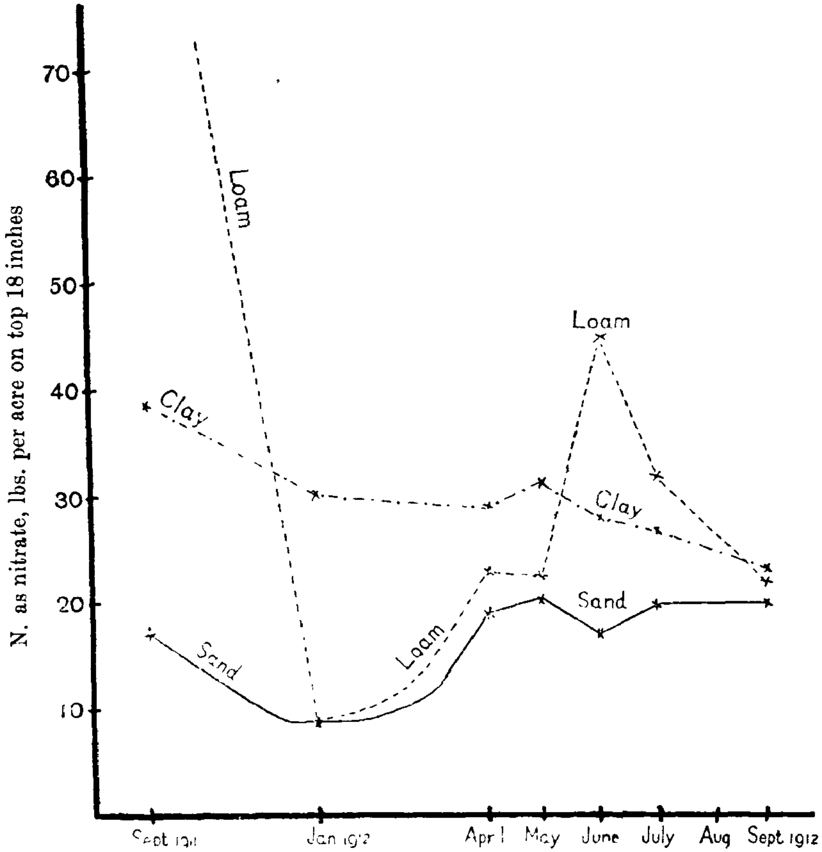

Fig. 1. Effect of soil type on accumulation of nitrates.

dunged plots at Rothamsted always containing more nitrate in winter than those receiving artificial fertilisers only.

It will be observed that the amounts of nitrogen as nitrate do not usually exceed certain limits, which in these experiments were :—

	Parts per million	lbs. per acre 0—18″
Sand	6	28
Loam	23	115
Clay	14	60

The heavily dunged loams, however, sometimes contained as much as 37 parts per million.

This limit to the accumulation of nitrate in soil has already been noticed in an earlier paper[1].

Effect of Season. Unfortunately it is not possible to characterise climatic factors with sufficient exactness for precise discussion, but some interesting results are obtained from a general comparison of the different seasons. The summer and autumn of 1909 and 1912 were cold and wet; those of 1911 were hot and dry. Table I shows that the amounts of nitrate present in 1911 are distinctly higher than in 1909 and 1912. In the latter years there was no tendency for nitrates to accumulate in autumn; in 1911, on the other hand, there was a well marked tendency in this direction, especially on two of the loams. Indeed at Rothamsted the nitrates attain in September 1911 the extraordinary high level of 115 lbs. per acre or 22 parts per million. Thus a hot dry summer favours the accumulation of nitrates on loams in autumn, a cold wet summer does not. These relationships are shown in Fig. 2. In the dry but cool summer of 1913 the maximum was attained in June (on one or two plots in July) and practically no change set in till the winter leaching began.

The rule does not hold universally on sandy soils. Of the four sand plots studied three showed no gain in nitrate during or after the hot weather, and only the fourth (No. 21) behaved like the loams and clays:—

N. as nitrate, lbs. per acre, 0—18 ins. in 1911.

No.	May 22	June 15	July 13	Aug. 9	Sept. 7
23	26	13	12·5	15	17
25	21	18	15	14·5	14
27	24·5	16	10	16	10
21	23	12	12	29	27

The soils, however, were very dry, the moisture steadily falling from 5 to 1·3 per cent. On the clay soil at Ridgmont no rise of nitrates was observed in autumn.

[1] This *Journal*, 1913, **5**, 197.

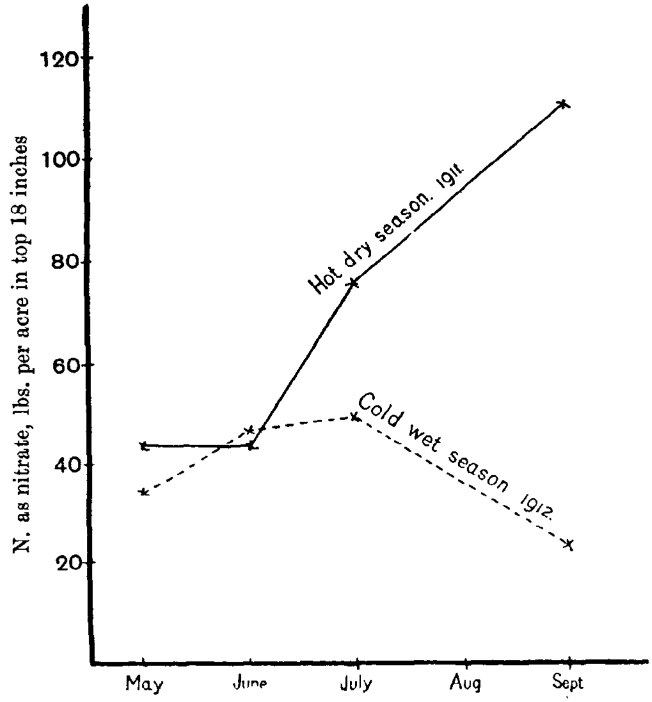

FIG. 2. Effect of season on accumulation of nitrates on the loam at Rothamsted.

TABLE I. *N. as nitrate, parts per million of dry soil. Soil uncropped except for fruit trees.*

A. Sand, Milbrook (no manure applied during the whole period)

1909	March	May	July	Sept.		
0—9″	5	8	3	2		
9—18″	4	5	3	2		
1910	**June**	**Aug.**	**Sept.**			
0—9″	6	4	5			
9—18″	5	3·5	3			
1911	**May**	**June**	**July**	**Aug.**	**Sept.**	
0—9″	5	3	2·5	4	4	
9—18″	4	3	2	3	2	
1912	**Jan.**	**April**	**May**	**June**	**July**	**Sept.**
0—9″	2	5	4	3	3	4
9—18″	1	2	3	3	4	3

C. Clay, Ridgmont (artificial manures applied in March or April containing 16 lbs. N. as $NaNO_3$, i.e. 7 parts per million)

1909	March	May	July	Sept.		
0—9″	8	7	4	5		
9—18″	12	5	4	4		
1910	**June**	**Aug.**	**Sept.**			
0—9″	7	5	9			
9—18″	7	5	5			
1911	**May**	**June**	**July**	**Aug.**	**Sept.**	
0—9″	11	14	10	6	11	
9—18″	9	11	6	5	5	
1912	**Jan.**	**April**	**May**	**June**	**July**	**Sept.**
0—9″	6	5	6	8	6	6
9—18″	6	7	7	4	5	4

TABLE I (*cont.*).

Loams I and II, Ridgmont (artificial manures applied in March or April containing 16 lbs. N. as $NaNO_3$, i.e. 7 parts per million)

1909	March	May	July	Sept.		
Loam I						
0—9″	5	15	2	3·5		
9—18″	4	7	1·5	2		
Loam II						
0—9″			4	5		
9—18″			5	3		
1910	June	Aug.	Sept.			
Loam I						
0—9″	3	3	1			
9—18″	2	2	3			
Loam II						
0—9″	7	12	12			
9—18″	4	6	7			
1911	May	June	July	Aug.	Sept.	
Loam I						
0—9″	11	11	8	6	6	
9—18″	6	5	6	3	4	
Loam II						
0—9″	16	19	15	15	18	
9—18″	9	12	8	8	11	
1912	Jan.	April	May	June	July	Sept.
Loam I						
0—9″	1	3	5	6	4	3·5
9—18″	1·5	3	3	6	2	2
Loam II						
0—9″	1	4	4	10	6	3
9—18″	1·5	3	3	4	4	4

Rothamsted (no manure applied)

1909	April	May	July	Oct.		
0—9″	5	12	6	5		
9—18″	4	6	6	7		
1910	May	July	Sept.			
0—9″	13	16	8			
9—18″		6	8			
1911	May	June	July	Sept.		
0—9″	10	8·5	17	23		
9—18″	7	8	12	22		
1912	Feb.	April	May	June	July	Sept.
0—9″	7	4	7	9	11	5
9—18″	5·5	3	6	8·5	7	4

NOTE.—During the experimental period the following nitrogenous manures were applied:—

Sand—none.

Clay and Loam (Ridgmont) 97 lbs. $NaNO_3$ (containing 16 lbs. N.) per acre on
March 20, 1909 (i.e. 7 days *after* the samples were taken),
March 11, 1910,
April 12, 1911,
April 24, 1912 (i.e. 9 days *before* the May samples were taken).

TABLE I (*cont.*). *N. as nitrate, lbs. per acre in dry soil,* 0—18 *ins.*

A. Sand, Milbrook (no manure applied during the whole period)

1909	March	May	July	Sept.		
	25	36	17	11		
1910	June	Aug.	Sept.			
	28	20·5	23			
1911	May	June	July	Aug.	Sept.	
	25	15	13	18	17	
1912	Jan.	April	May	June	July	Sept.
	9	19	20	17	20	20

C. Clay, Ridgmont (artificial manures containing 16 lbs. N. as $NaNO_3$ applied in March or April)

1909	March	May	July	Sept.		
	47	30	20	22		
1910	June	Aug.	Sept.			
	35	24	33			
1911	May	June	July	Aug.	Sept.	
	47	60	38	26	39	
1912	Jan.	April	May	June	July	Sept.
	30	29	31	28	27	23

[illegible] in March or April)

1909	March	May	July	Sept.		
Loam I	28	69	11	17		
Loam II			30	27		
1910	June	Aug.	Sept.			
Loam I	13	17	8			
Loam II	34	55	59			
1911	May	June	July	Aug.	Sept.	
Loam I	51	50	43	29	32·5	
Loam II	79	95	73	69	90	
1912	Jan.	April	May	June	July	Sept.
Loam I	9	20	24	30·5	19	17
Loam II	8·5	23	22	45	32	22

Rothamsted (no manure applied)

1909	April	May	July	Oct.		
	21	46	31	31		
1910	May	July	Sept.			
	34	56	42			
1911	May	June	July	Sept.		
	43·5	43	75	115		
1912	Feb.	April	May	June	July	Sept.
	31	18	34	45·5	48	23

NOTE.—In reducing parts per million to lbs. per acre the following weights of fine earth (dry) in millions of lbs. per acre were taken:—

	Milbrook sand	Ridgmont clay	Ridgmont loam
0—9″	2·80	2·45	2·99
9—18″	2·58	2·35	3·35

These figures were kindly supplied by Mr Pickering.

Rothamsted soils.

	Broadbalk, Orchard	Broadbalk plots: Dunged plot	Broadbalk plots: Others	Hoosfield barley plots: Dunged plot	Hoosfield barley plots: Others	Hoosfield wheat, fallow plot
0—9″	2·58	2·51	2·59	2·08	2·53	2·65
9—18″	2·56	2·67	2·67	2·59	2·59	2·70

TABLE II. *Effect of manures on nitrate content.*

Uncropped land. Season 1912. Hoosfield Barley plots left fallow. Nitrogen as nitrate.

Nitrogen as nitrate, parts per million of dry soil

Usual Manure	Plot No.	Feb. 15		Mar. 12		Mar. 26		Apr. 10		Apr. 19		Apr. 30		May 13		May 22		June 26		July 22		Sept. 26		Feb. 4, '13	
		0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9′	9—18″	0—9″	9—18″
No manure..................	Fallow	6	5	4	5	5	3	5	5	7	4	5	5	5	4	4	4	9	7	8	10	5	3	4	4
Amm. salts..................	1 A	5	5	5	4	4	3	6	5	8	7	8	6	7	5	10	7	14	11	16	11	6	6	4	4
Do. + complete minerals	4 A	5	6	4	6	6	4	7	7	6	7	7	6	7	6	6	4	9	12	14	9	7	8	3	3
Dung	7^2	9	7									15	15	18	12	12	13	28	19	30	19	16	19	6	6

lbs. per acre, 0—18″

Usual Manure	Plot No.	Feb. 15	Mar. 12	Mar. 26	Apr. 10	Apr. 19	Apr. 30	May 13	May 22	June 26	July 22	Sept. 26	Feb. 4, '13
		0—18″ lb.	0—18″ lb.	0—18′ lb.	0—18″ lb.	0—18″ lb.	0—18″ lb.	0—18″ lb.	0—18″ lb.	0—18″ lb.	0—18″ lb.	0—18″ lb.	0—18″ lb.
No manure..................	Fallow	28	24	19	25	29	27	23	22	39	45	19	20
Amm. salts..................	1 A	25	22	17	30	37	38	31	43	64	71	28	19
Do. + complete minerals	4 A	28	26	25	34	33	32	33	25	56	58	36	16
Dung	7^2	38					69	68	58	105	110	83	27

The plots being fallowed this year no manure was applied during the whole period; the last lot of ammonium salts were put on for the preceding crop in Feb. 1911, and the last dung (14 tons) was applied to 7^2 at the same time.

TABLE II (*cont.*).

Cropped land. Wheat season, 1912. Broadbalk. Nitrogen as nitrate.

Parts per million of dry soil

Usual Manure	Plot No.	Feb. 29		Mar. 12		Mar. 22		Apr. 10		Apr. 19		Apr. 30		May 13		May 22		June 26		Sept. 26		Feb. 4, '13	
		0—9"	9—18"	0—9"	9—18"	0—9"	9—18"	0—9"	9—18"	0—9"	9—18"	0—9"	9—18"	0—9"	9—18"	0—9"	9—18"	0—9"	9—18"	0—9"	9—18"	0—9"	9—18"
No manure...........	3	4	4	4	5	4	3	5	6	5	5	—	—	4	5	7	9	5	1	5	3	3	4
Double amm. salts	10	7	5	5	2	4	3	10	8	9	10	12	7	14	16	31	17	19	12	4	6	3	4
Do. + minerals	7	6	8	6	6	4	4	9	4	7	3	13	10	8	9	17	15	9	—	8	6	6	6
Dung	2	12	9	16	4	6	8	10	10	11	9	4	8	12	11	16	16	10	8	10	6	10	7

lbs. per acre 0—18"

Usual Manure	Plot No.	Feb. 29	Mar. 12	Mar. 22	Apr. 10	Apr. 19	Apr. 30	May 13	May 22	June 26	Sept. 26	Feb. 4, '13	Nitrogen in crop, lbs. per acre
		lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	
No manure...........	3	23	24	17	27	26	—	23	42	17	21	20	7·6
Double amm. salts...	10	31	21	18	48	51	49	77	124	81	27	18	6·2
Do. + minerals	7	35	32	22	35	26	61	46	83	24	38	31	13·1
Dung	2	54	50	35	49	48	31	56	82	44	40	41	32·3

• The ammonium salts were applied in two dressings, one fourth (containing 21·5 lbs. N) on Sept. 26—27, 1911, and the remainder (containing 64·5 lbs. N) on March 27, 1912. The dung (14 tons, containing 200 lbs. N) was applied on Sept. 15, 1911.

TABLE II (*cont.*).

Season 1909. Broadbalk Wheat plots. Nitrogen as nitrate.

Manure	Plot No.	Parts per million of dry soil 0—9″ Apr. 6	May 7	July 6	Oct 28	lbs. per acre 0—9″ Apr. 6	May 7	July 6	Oct. 28
						lb.	lb.	lb.	lb.
Double amm. salts	10	6	16	3	6	14	42	8	16
Do. + minerals	7	8	16	3	10	21	42	9	27

Ammonium salts applied one quarter (21·5 lbs. N) on Oct. 7, 1908, and the rest (64·5 lbs. N) on April 7, 1909.

Season 1909. Hoosfield Barley plots. Nitrogen as nitrate.

Manure	Plot No.	Parts per million of dry soil 0—9″ Apr. 6	May 7	July 6	Oct. 28	lbs. per acre 0—9″ Apr. 6	May 7	July 6	Oct. 28
						lb.	lb.	lb.	lb.
Unmanured	1 O	6	—	3	3	15	—	7	8
Amm. salts	1 A	13	9	2	3	33	23	4	9
Super + amm. salts......	2 A	—	13	3	3	—	33	8	7
Amm. salts + complete minerals	4 A	15	10	3	3	37	26	9	8
Dung	7²	22	21	4	6	46	43	7	12

Ammonium salts (43 lbs. N) applied Feb. 20, 1909, and dung (200 lbs. N) on Feb. 22, 1909.

Season 1911.

Manure	Plot No.	Parts per million of dry soil: May 17 0—9″	May 17 9—18″	June 8 0—9″	June 8 9—18″	Sept. 13 0—9″	lbs. per acre: May 17	June 8	Sept. 13	Nitrogen in crop, lbs. per acre
							lb.	lb.	lb.	
Unmanured	1 O	7	7	6	4	7	35	25	18	8
Amm. salts	1 A	7	8	6	10	8	40	42	20	21
Super + amm. salts	2 A	12	14	11	12	7	67	59	18	24
Amm. salts + complete minerals ...	4 A	12	14	9	10	5	67	49	13	34
Dung	7²	17	7	12	13	9	53	58	18	37

Ammonium salts (43 lbs. N) applied Feb. 18, 1911, and dung (200 lbs. N) on Feb. 14, 1911.

TABLE II (*cont.*).

Season 1910. Barnfield Mangolds. (These being sown late the land is practically fallow till the middle of June.)

Manure	Parts per million of dry soil 0—9″ June 4	Parts per million of dry soil 0—9″ July 27	lbs. per acre 0—9″ June 4	lbs. per acre 0—9″ July 27
Unmanured	4·5	3	12	8
Super only	6·5	2	17	5
Super + potassium salts	5	2	13	5
Super + magnesium and sodium salts	5	2	13	5
Super + potassium, mag. and sodium salts	4	2·5	10	7
Dung*	24·5	7	56	16
Dung + potassium salts *	37	4	85	9

* The dung was applied April 11, 1910.

Losses during summer and winter. A wet autumn or winter has a disastrous effect on the nitrates in loams and sands. At the end of the fine season of 1911 a considerable store of nitrate had accumulated, but a great proportion was lost during the wet winter that followed :—

	Rainfall during period, ins.	N. as nitrate: Parts per million				N. as nitrate: lbs. per acre	
		Broadbalk orchard		Hoos fallow		Broadbalk orchard	Hoos fallow
		0—9″	9—18″	0—9″	9—18″	0—18″	0—18″
Sept. 13, 1911	19·91	23	22	13	7	115	51
Feb. 15, 1912		7	5	5	4	31	28
		Ridgmont loam (105)		Milbrook sand (21)		Ridgmont loam (105)	Milbrook sand (21)
Sept. 7, 1911	11·67	17	14	6	4	97	29
Jan. 15, 1912		2	1	2	2	7	10

The loss, however, was much less on the clay soil :—

	Parts per million 0—9″	Parts per million 9—18″	lbs. per acre
Sept. 7, 1911	19	9	67
Jan. 13, 1912	11	12	56

Equally serious losses occur in a wet autumn. The results obtained in 1912 were :—

	Rainfall in intervening period	N. as nitrate, parts per million in uncropped land. Hoosfield *, 1912.							
		Dung 7[2]		Artificial manures				Unmanured †	
				1 A		4 A			
		0—9″	9—18″	0—9″	9—18′	0—9″	9—18″	0—9″	9—18″
Summer, July 22		30	19	16	11	14	9	8	10
Autumn, Sept. 26	9·00	16	19	6	6	7	8	4	3
Late winter, Feb. 4	14·70	6	6	4	4	3	3	4	3

* The Hoosfield barley plots which were fallowed during the season of 1912 (see p. 27).
† The fallow portion of the wheat plots.

	N. as nitrate, lbs. per acre			
	Dung 7[2]	Artificial manures		Unmanured
		1 A	4 A	
	0—18″	0—18″	0—18″	0—18″
Summer, July 22	110	71	58	45
Autumn, Sept. 26....	83	28	36	19
Late winter, Feb. 4	27	19	16	20

The amount of loss depends on the wetness of the period. The driest winter during the period was that of 1908—09, and the wettest was that of 1911—12. We find higher values for the nitrates in all the soils in March 1909 than in April 1912. The wetness of the winter may be approximately estimated from the rainfall and the number of days on which the drain gauges ran ; the figures are as follows :—

Year	Wetness of preceding winter (Oct.—March)		Nitrate, lbs. per acre in top 18″ in spring			
	Rainfall in inches	No. of days on which drain gauges ran	Sand	Clay	Loam	
					Ridgmont	Rothamsted
1909	10·29	32	25	47	28	21
1912	23·56	80	19	29	21	18

The effect of the wet winter persisted and the May maximum of 1912 is lower than that of any other year.

2. *Cropped land.* The effect of a crop is to reduce considerably the amount of nitrate in the soil. On plots of cereals the amount usually falls from about May right through to the time of harvest; in the case of mangolds the fall begins rather later[1]. When a cropped plot is compared with a fallow plot there is a steadily increasing difference from spring to autumn, as shown in the following figures:—

Nitrate in adjoining fallow and cropped plots.

Broadbalk orchard*, 1911	May 7		June 8		July 27	
	A	B	A	B	A	B
Fallow, parts per mill., top 9″	11	10	10	7	21	15
9—18″	5	9	8	7	15	13
lbs. per acre, 0—18″.........	40	50	46	35	93	72
Cropped (Lucerne and grass) parts per million, top 9″ ...	6	8	4	4	8	11
9—18″...	6	5	2	4	5	9
lbs. per acre, 0—18″	31	32	16	20	35	49

* This land was a lucerne ley from 1905—1908, the part called fallow was then dug up and in 1909 planted with small fruit trees. A and B are at the extreme ends of the plot. No manure was added.

Hoosfield wheat plots, 1911	April 21	May 17	June 8	Sept. 13
Fallow, parts per mill., top 9″	10	7	12	13
9—18″	—	5	9	7
lbs. per acre, 0—18″.........	(27)*	33	55	54
Cropped (Wheat), parts per million, top 9″	9	6	4	5
9—18″...............	—	5	2	—
lbs. per acre, 0—18″.........	(23)*	30	15	13

* 0—9″ only.

[1] On June 4th, 1910, the dunged mangold plots 1—0 and 2—0 contained respectively 16 and 19 parts of nitrogen as nitrate per million of soil; by July 27th these amounts were reduced to 7 and 5 respectively.

1912	Feb. 15	Mar. 12	Mar. 26	Apr. 10	Apr. 19	Apr. 30	May 13	May 22	June 26	July 22	Sept. 26	Feb. 4, 1913
Fallow, parts per million, top 9″	6	4	5	5	7	5	5	4	9	8	5	4
9—18″	5	5	3	5	4	5	4	4	7	10	3	4
lbs. per acre, 0—18″	28	24	19	25	29	27	23	22	39	45	19	20
Cropped (Wheat), parts per million, top 9″	5	4	5	5	6	3	7	5	5	2	3	4
9—18″	4	6	3	3	3	4	5	4	4	3	3	4
lbs. per acre, 0—18″	24	25	19	22	24	18	31	24	22	13	16	21

No manure is applied to these Hoosfield plots.

The difference during June and July is seen to be great; the cropped plots in Broadbalk contained 23 and 58 lbs. per acre less than the fallow land, and that in Hoosfield contained 40 lbs. less in 1911 and 32 lbs. in 1912.

Thus the cropped land tends to contain a minimum amount of nitrate at harvest time and there is less difference than might be expected in different seasons on the same land. The results obtained by Warington[1] in October 1881 and October 1893 are of the same order as those obtained on the same plots in 1912:—

Plot		N. as nitrate in lbs. per acre, 0—18″		
		Oct. 1881	Oct. 1893	Sept. 26, 1912
3 & 4	Unmanured	14	17	21
10	Ammonium salts only	24	38	27
7	Amm. salts + complete minerals	32	34	38
2 B	Dung	44	56	40

After the harvest the amounts of nitrate may rise: thus in 1909 two of the Broadbalk plots yielded the following results in comparison with a fallow unmanured plot:—

[1] Warington, R., "Lost Fertility: the Production and Loss of Nitrates in the Soil," *Trans. Highland Agric. Soc.* 1905, **17** (5th series), 148-181.

	Parts per million					lbs. per acre				
	April, 0—9″	May, 0—9″	July, 0—9″	Oct., 0—9″	Oct., 9—18″	April, 0—9″	May, 0—9″	July, 0—9″	Oct., 0—9″	Oct., 0—18″
Cropped plots										
Plot 10 Ammonium salts only.........	6	16	3	6	3	14	42	8	16	—
,, 7 Ammonium salts + complete minerals	8	16	3	10	3	21	42	9	27	36
Fallow plot										
Young fruit trees only, no manure......	4	8	6	5	7	11	20	16	14	31

In July the cropped plots contained very little nitrate, only about half as much as the uncropped plot. But a month after the harvest was over the nitrate had increased so much in amount that it exceeded that present on the uncropped plot, there being 36 lbs. per acre in the top 18 inches against 31 lbs. Similar increases were obtained in 1913 on the dunged but not on the unmanured plots:—

N. as nitrate, parts per million

	Before harvest	After harvest 17 days	After harvest 6 weeks
Broadbalk plots			
Unmanured	6	8	6
Dunged............	7	14	17

The fate of the nitrates on the cropped plot. The preceding paragraphs have shown the marked difference in nitrate content between a cropped and an uncropped piece of land. One obvious reason for the difference is that the crop absorbs some of the nitrate. It does not appear, however, that this is the sole factor involved, for even after allowing for the nitrogen in the crop there is still a deficit as compared with the fallow land. This is well seen on Hoosfield, where two adjacent strips of land are alternately cropped with wheat and left fallow for a year, both being unmanured. The nitrate found on these strips in lbs. per acre was as follows:—

	In 1911		In 1912	
	Fallow land	Cropped land	Fallow land	Cropped land
N. as nitrate in top 18″ of soil, June	53·7	15·3	46·1	12·8
Nitrogen in crop		22·6*		6·1*
Total.........	53·7	37·9	46·1	18·9
Deficit in cropped land		15·8		27·2

* The nitrogen in the crop was obtained as follows:—

	Straw			Grain			Total N. in straw and grain, lbs. per acre
	Crop, lbs. per acre	N., per cent.	N., lbs per acre	Crop, lbs. per acre	N., per cent.	N., lbs. per acre	
1911	1535	0·36	4·7	1152	1·78	17·9	22·6
1912	706	0·43	2·4	266	1·67	3·7	6·1

It is impossible to say how much of the excess of nitrate on the fallow land of 1911 arises from the stubble left over from 1910, when the cropped land of 1911 was lying fallow and therefore carrying no stubble. So with the fallow strip of 1912, which had been the cropped strip of 1911. But the whole straw only contains 3 or 4 lbs. of nitrogen, and the stubble certainly contains no more than this if as much, while the deficit to be accounted for is 16 lbs. in 1911 and 27 lbs. in 1912. Nor does leaching account for the difference, because the loss by leaching is greatest on the fallow strip. Whatever the explanation, the fact remains that cropped land contains less nitrate by the end of the season than fallow land, even after allowance has been made for the nitrate taken up by the crop.

We can even go further. No evidence could be obtained that the cropped soils gained in nitrates after the early summer. When the nitrogen as nitrate contained in the top 18 inches of soil at the end of the season (Aug. or Sept.) is added to that in the crop the sum is not more than the stock present in May. The following results were obtained in 1911 on the Hoosfield barley plots:—

	Ammonium salts only, 1 A		Dung 7[2]	
Nitrate in soil in May	40		53	
,, ,, Sept.		20		18
Nitrogen in crop		21		37
Balance to be accounted for	1		2	
Total	41	41	55	55

Here the amount of nitrogen accounted for in September is approximately equal to the amount present in May. But in other cases it is not even equal; the September amounts do not balance those found in May and some other source of loss must come into play to account for the deficit:—

	Hoos Barley, 1911						Hoos wheat, 1912 Unmanured	
	Unmanured		Amm. salts + super, Plot 2 A		Amm. salts + complete minerals, Plot 4 A			
Nitrate in soil (0—18″) in May	35		67		67		31	
,, ,, ,, Sept.		18		18		13		16
Nitrogen in crop		8		24		34		6
Balance to be accounted for		9		25		20		9
Total	35	35	67	67	67	67	31	31

	Broadbalk wheat, 1912						Dung, Plot 2 b	
	Unmanured, Plot 3		Amm. salts alone, Plot 10		Amm. salts + complete minerals, Plot 7			
Nitrate in soil (0—18″) in May	42		124		83		82	
,, ,, ,, Sept.		21		27		38		40
Nitrogen in crop		8		6		13		32
Balance to be accounted for		13		91		32		10
Total	42	42	124	124	83	83	82	82

In 1912 the Broadbalk plots were exceedingly foul and an unknown fraction of the missing nitrogen was removed in the weeds. Owing to the wetness of the season a certain amount of leaching took place also. It is less easy to account for the loss on Hoosfield in 1911. The important point, however, is that in no case is there more nitrate in the soil *plus* crop in autumn than there was in spring, and generally there is less.

It cannot be argued from these results that an actual loss of nitrate is brought about by the activity of the plant roots because we have no certain knowledge that the barley roots can forage so deeply as 18 inches, the depth to which the nitrate has been taken into account. Indeed if we confine ourselves to a depth of 9 inches then the crop usually obtains more than is lost from this thickness of soil :—

	Broadbalk, 1909 Amm. salts + complete minerals		Hoos barley plots, 1909						Dung 7^2	
			Un-manured, Plot 1—0		Amm. salts + super, Plot 2 A		Amm. salts + complete minerals, Plot 4 A			
Nitrate in soil (0—9″) in May...	42		15		33		37		46	
,, ,, ,, Sept...		9		7		8		9		7
Nitrogen in crop		44		14		38		48		71
Balance to be accounted for ...	11		6		13		20		32	
Total.........	53	53	21	21	46	46	57	57	78	78

The Hoos barley plots, 1—0, 1—A, and 7^2, generally give similar results. In a few cases (Hoos barley 1 A of 1909 and 2 A of 1910) the amount in the crop almost equals the quantity lost from the soil, but on Broadbalk Plot 10 it is less :—

	Broadbalk, 1909 Amm. salts only (Plot 10)		Hoos barley plots			
			1909 Amm. salts only (Plot 1 A)		1910 Amm. salts + super (Plot 2 A)	
Nitrate in soil (0—9″) in May...	42		33		42	
,, ,, ,, July...		8		4		9
Nitrogen in crop		22		27		30
Balance to be accounted for ...		12		2		3
Total.........	42	42	33	33	42	42

The results may be briefly summed up as follows:—The quantity of nitrate found in the top 18 inches of a cropped soil in late summer was no greater than was present in late spring even after allowing for what had been taken by the crop. This is not true of fallow soils; here more nitrate was found in late summer than late spring even in a bad year such as 1912 (Table II). The significance of these results will be discussed later.

Effect of manuring. As already stated, nitrate production is the quickest of the stages in the decomposition of organic matter so that ammonia does not accumulate in the soil under normal conditions. But the case is rather different when ammonium salts are added to the soil. Part of the ammonia enters into some stable combination from which it is not dislodged by heating with magnesia[1], but much of it remains as an ammonium compound and can be detected in the usual way; this part survives for some time. Ammonium salts applied in the February of 1909 were not completely converted into nitrate even at the end of seven weeks, but quantities applied later on (April 5th) were practically completely nitrified in four weeks:—

Plot	Date of application of amm. salts	N. present as NH_3 after			N. present as nitrate after		
		44 days (April 6)	75 days (May 7)		44 days (April 6)	75 days (May 7)	
Hoos barley 4 A	Feb. 20 and 21, 1909	13	2		15	10	
1 A	,,	4	2		13	9	
		1 day (Apr. 6)	32 days (May 7)	92 days (July 6)	1 day (Apr 6)	32 days (May 7)	92 days (July 6)
Broadbalk 7	April 5, 1909	19	5	4	8	16	3
,, 10	,, ,,	13	3	1·5	6	16	3

Parts per million of dry soil.

In 1912 the process was slower and ammonium salts added on March 27 were still yielding nitrates in the latter half of May (Table II).

Effect of potassic and phosphatic manures. Determinations have been made on the plots which for many years have received neither potassium salts nor phosphates to ascertain whether a deficiency of

[1] Russell, this *Journal*, 1910, **3**, 241.

these substances would retard the process of nitrification. Reference to Table II shows that such an effect was produced only in 1911; in the other years there is nothing to show that these particular organisms are affected although plants suffer to a marked degree from potash and phosphate starvation. Indeed in 1912 and 1913 there was more nitrate on the plots supplied with ammonium salts only than where potassium salts and phosphates are given. In 1912 the land lay fallow, but in 1913 it carried a barley crop which however was much larger on the completely fertilised plot than on the others.

Residual effect of ammonium sulphate. In 1912 the Hoosfield plots were fallowed and no manures were applied. It was found that the plots which regularly receive ammonium salts contained a higher proportion of nitrate than the unmanured plot (Table II). To some extent this may arise from the decomposition of the stubble which, being increased in amount by addition of ammonium salts, yields larger amounts of nitrate on decomposition. There appears, however, to be some other factor involved, because Plot 1 A, receiving ammonium salts only, contained *more* nitrate than Plot 4 A, which receives in addition potash and phosphates, although it always has a *smaller* crop. In the preceding year (1911) it had contained less nitrate than 4 A besides yielding a smaller crop. These results strongly suggest that some of the ammonium salts had been held over in some form or other from 1911 till 1912.

The possibility of such an action has been investigated on the Broadbalk wheat field[1]. Two plots (Nos. 17 and 18) side by side receive ammonium salts only in alternate years, so that each year one has a dressing and the other has not. A third plot (No. 5) receives the same mineral manures but no nitrogen manure at any time. The average results for the past sixty years have been :—

Plots		Total produce, lbs. per acre	Grain, bushels per acre
17 & 18	Years when ammonium salts are supplied ...	1354	29·9
	Years when no ammonium salts are supplied	842	14·9
5	No nitrogenous manure since 1852	799	14·5

Thus the omission of ammonium salts for a single year brings down the crop nearly to the level of Plot 5 showing that only a small residue

[1] A similar experiment has been made at Woburn and a distinct residual effect is obtained.

is left behind from the preceding year. Nitrate determinations lead to the same conclusion. The amounts of nitrogen as nitrate found on April 18th, 1913, were :—

Plots		Parts per million		lbs. per acre, 0—18″
		0—9″	9—18″	
5	Mineral manures only	4·5	5	25
17	Mineral manures + amm. salts in March, 1912	6·5	6	33
18	,, ,, ,, ,, 1913	11	5	42

The differences here are much smaller than in Hoosfield.

The Effect of Fluctuations in Nitrate Content on the Crop.

The relation between the amount of nitrate in the soil and the amount of crop growth is in the main fairly simple. So long as there is sufficient potash and phosphate in the soil the crop increases with the nitrate supply until some limiting factor (such as water supply, temperature, etc.) intervenes and puts an end to further growth. The factors that regulate the amount of nitrate in the soil are therefore controlling factors in crop production. Since, however, their action is on the nitrate and not on the crop we cannot expect to find them closely and immediately related to the amount of crop growth: nevertheless over an average of years their action is plainly indicated.

The foregoing experiments have brought out the following conclusions :—

1. Nitrates are rapidly produced in spring or early summer.

2. Unless absorbed by a crop they remain in the soil and tend to increase in amount during a dry summer.

3. They are more largely removed during a wet winter than in a dry winter.

4. Nitrates are present in greatest amount in spring when the preceding winter and summer have been dry: they are on the other hand present in smaller quantity when the preceding winter has been wet. If the preceding summer has been dry so that nitrate accumulation went on to a considerable extent the loss during winter is proportionately greater.

A study of the Rothamsted data shows that the operation of these relationships can be readily traced in the growth of the crop. In particular the loss of nitrates during a wet winter has a marked effect, as shown in Tables III and IV. Two sets of experiments illustrate

TABLE III. *Effect of Autumn and Winter rainfall on Soil Nitrates as illustrated by yield of Wheat. Broadbalk Field.*

Comparison of Spring and Autumn dressings of ammonium salts.

Year when crop was reaped	Rainfall of preceding autumn and winter (Oct. to March)	Total produce, lbs. per acre				Grain, bushels per acre			
		Ammonium salts applied		Difference in favour of spring dressing		Ammonium salts applied		Difference in favour of spring dressing	
		in previous autumn Plot 15	in spring Plot 7	per acre	per cent.	in previous autumn Plot 15	in spring Plot 7	per acre	per cent.
(A) Years of LOW winter rainfall									
1889	12·35	4422	5149	+ 727	+16·4	27·0	30·7	+3·7	+13·7
1890	12·83	6631	5806	− 825	−12·4	40·8	36·0	−4·8	−11·8
1891	8·98	6633	7596	+ 963	+14·5	38·9	40·6	+1·7	+ 4·3
1893	13·76	2377	2614	+ 237	+ 9·9	18·5	20·3	+1·8	+ 9·7
1898	8·46	7043	6924	− 119	1·6	30·0	28·4	−1·6	− 5·3
1901	13·85	4579	4339	− 240	− 5·2	29·8	29·6	−0·2	− 0·6
1902	10·76	6560	6727	+ 167	+ 2·5	39·6	38·2	−1·4	− 3·5
1903	12·28	3221	4817	+1596	+49·5	20·2	26·6	+6·4	+31·7
1905	11·37	7285	8037	+ 752	+10·3	37·5	40·7	+3·2	+ 8.5
1906	14·05	6984	6238	− 746	−10·6	42·2	37·7	−4·5	−10·6
1909	10·29	6212	5867	− 345	− 5·5	25·8	28·9	+3·1	+12·0
Mean	11·73	5631	5829	+ 196	+ 3·4	31·8	32·5	+0·7	+ 2·2
(B) Years of HIGH winter rainfall									
1892	16·85	4702	5240	+ 538	+11·4	28·6	32·0	+3·4	+11·8
1894	16·54	7677	9144	+1467	+19·1	40·4	48·4	+8·0	+19·8
1895	14·94	3517	4519	+1002	+28·4	25·1	32·2	+7·1	+28·2
1896	15·45	4977	6204	+1227	+24·6	30·8	37·2	+6·4	+20·7
1897	19·08	3478	5030	+1552	+44·6	20·5	28·6	+8·1	+39·5
1899	14·61	5283	6503	+1220	+23·0	26·9	31·3	+4·4	+16·3
1900	18·45	3281	4793	+1512	+46·0	20·2	29·8	+9·6	+47·5
1907	16·34	7654	8516	+ 862	+11·2	33·1	33·6	+0·5	+ 1·5
1908	17·04	4995	5607	+ 612	+12·2	32·3	33·3	+1·0	+ 3·0
1910	17·04	4533	5633	+1100	+24·2	20·4	25·6	+5·2	+25·4
1911	17·63	4154	4854	+ 700	+16·8	24·1	25·6	+1·5	+ 6·2
Mean	16·73	4932	6004	+1072	+21·7	27·5	32·5	+5·0	+18·1

TABLE IV. *Effect of Winter rainfall on Soil Nitrates as illustrated by yield of Wheat.*

Comparison of wheat grown continuously with wheat grown after fallow.

Year when crop was reaped	Rainfall of preceding autumn and winter (Oct. to March)	Total produce, lbs. per acre				Grain, bushels per acre			
		Unmanured		Difference in favour of growing after fallow		Unmanured		Difference in favour of growing after fallow	
		Plot 3 Broadbalk, continuous	Plot O after fallow	per acre	per cent.	Plot 3 Broadbalk, continuous	Plot O after fallow	per acre	per cent.
(A) Years of LOW winter rainfall									
1889	12·35	1645	1712	+ 67	+ 4·1	12·2	13·1	+0·9	+ 7·4
1890	12·83	1853	2745	+ 892	+ 48·1	14·0	17·8	+3·8	+27·1
1891	8·98	2143	3645	+1502	+ 70·1	13·8	23·1	+9·3	+67·4
1893	13·76	1251	1724	+ 473	+ 37·8	9·8	13·5	+3·7	+37·8
1898	8·46	2186	3964	+1778	+ 81·3	12·0	20·2	+8·2	+68·3
1901	13·85	1627	2062	+ 435	+ 26·7	11·7	14·7	+3·0	+25·6
1902	10·76	1853	3729	+1876	+101·2	13·3	22·4	+9·1	+68·4
1903	12·28	1078	2111	+1033	+ 95·8	7·6	14·0	+6·4	+84·2
1905	11·37	3456	2596	− 860	− 24·9	18·0	12·9	−5·1	−28·3
1906	14·05	2156	2340	+ 184	+ 8·5	15·2	13·4	−1·8	−11·8
1909	10·29	1633	2326	+ 693	+ 42·4	9·1	12·9	+3·8	+41·8
Mean	11·732	1898	2632	+ 734	38·7	12·4	16·2	+3·8	+30·6
(B) Years of HIGH winter rainfall									
1892	16·85	1425	1839	+ 414	+ 29·1	9·3	11·7	+2·4	+25·8
1894	16·54	2608	2436	− 172	− 6·6	18·0	15·5	−2·5	−13·9
1895	14·94	1384	2129	+ 745	+ 53·8	10·0	15·5	+5·5	+55·0
1896	15·45	2396	2332	− 64	− 2·7	16·8	16·1	−0·7	− 4·2
1897	19·08	1459	1170	− 289	− 19·8	8·8	7·1	−1·7	−19·3
1899	14·61	1825	2620	+ 795	+ 43·6	12·0	15·7	+3·7	+30·8
1900	18·45	1776	1801	+ 25	+ 1·4	12·3	11·9	−0·4	− 3·3
1907	16·34	1715	3094	+1379	+ 80·4	9·1	14·3	+5·2	+57·1
1908	17·04	1671	1083	− 588	− 35·2	12·4	7·2	−5·2	−42·0
1910	17·04	1553	1747	+ 194	+ 12·4	7·5	9·3	+1·8	+24·0
1911	17·63	1935	2687	+ 752	+ 38·9	12·5	17·0	+4·5	+36·0
Mean	16·73	1795	2085	+ 290	+ 16·2	11·7	12·8	+1·1	+ 9·4

this point very clearly; one on Broadbalk where the effect of autumn application of sulphate of ammonia is contrasted with that of spring

applications, and the other in Hoosfield, where a wheat crop is taken after a bare fallow. Plots 7 and 15 on the Broadbalk wheat field each receive the same complete mineral and ammonium salts, but on Plot 7 the ammonium salts are applied in spring and on Plot 15 they are applied in autumn. In years of low winter rainfall there is on an average practically no difference in yield, but in years of high winter rainfall the autumn dressings give considerably poorer results than the spring dressings.

The Hoosfield experiment enables us to compare the yield of wheat on land where it grows every year with that on land where it grows alternate years only, the intervening years being fallow. When there is no crop on the ground the nitrates accumulate to a notable extent (see p. 32), and if they remain till the following spring they increase the yield of wheat over and above what is obtained under continuous cropping. But if the winter has been wet much of the advantage is lost and the difference between the plots becomes considerably less. The data are given in Table IV, and it is seen that on an average after dry winters the crop preceded by a fallow is 38 per cent. higher than that preceded by another crop, but after wet winters it is only 16 per cent. higher.

A hot dry summer followed by a mild wet winter is, as we have seen, unfavourable to nitrate accumulation. The data collected in Table V show that the crop is adversely affected. Of the two Broadbalk plots 2 B receives farmyard manure every year and the plant is therefore dependent on nitrification for its nitrogenous food, while 16 receives a complete artificial manure containing more than enough nitrate of soda to supply nitrogen for the plant. On an average over the whole period during which the years were selected (1874—1912) the crops on the two plots are almost alike, being 6374 lbs. of total produce per acre on Plot 2 B and 6540 on Plot 16. But when the season has been preceded by a dry winter and this in turn by a dry summer the returns from the dunged plot are at a maximum, averaging 7537 lbs. per acre against 6375 lbs. from the plot with artificial manures, a difference of 1162 lbs. per acre in favour of dung. This is when the conditions are favourable for nitrate accumulation. When the preceding winter has been wet and the summer dry, i.e. when the conditions become unfavourable for nitrate accumulation, the position is reversed: farmyard manure now gives a much inferior crop of 5709 lbs. per acre, while the artificial manures give 6635 lbs., a difference of 926 lbs. in favour of the artificial manures. The result of course cannot be wholly attributed

TABLE V. *Effect of winter rainfall after dry summers. Yield of wheat on Broadbalk field.*

ear when rop was reaped	No. of days the drain gauges ran during: summer of preceding year (July—Sept.)	No. of days the drain gauges ran during: preceding winter (Oct.—March)	Rainfall of: summer of preceding year (July—Sept.)	Rainfall of: preceding winter (Oct.—March)	Yield of wheat, total produce per acre, lbs.: Broadbalk Dunged 2 b	Broadbalk Plot 16 (complete artificials)	Difference in favour of dung	Broadbalk unmanured Plot 3 continuous	Hoosfield ½ acre after fallow	Difference in favour of fallow	Yield of grain, bushels per acre: Broadbalk Dunged 2 b	Broadbalk Plot 16 (complete artificials)	Difference in favour of dung	Broadbalk unmanured Plot 3 continuous	Hoosfield ½ acre after fallow	Difference in favour of fallow
							Crops preceded by dry summers and wet winters									
1874	14	124	7·41	9·84	6870	7121	− 551	1684	3370	1686	39·3	38·2	+ 1·1	11·4	21·5	10·1
1875	8	107	8·17	13·40	5609	6712	−1103	1575	2718	1143	28·8	30·5	− 1·7	8·6	16·1	7·5
1877	22	158	9·45	21·36	3747	6235	−2488	1291	1478	187	24·1	40·1	−16·0	8·8	10·5	1·7
1881	35	98	12·18	19·32	4274	5911	−1637	2009	1645	−364	30·2	35·3	− 5·1	13·7	12·3	−1·4
1882	23	87	9·74	16·06	5997	8286	−2289	1774	1804	30	32·7	31·8	+ 0·9	10·9	11·8	0·9
1883	15	108	6·11	21·77	5174	8032	−2858	1878	2461	583	35·2	43·3	− 8·1	13·8	18·1	4·3
1884	27	100	9·04	12·81	5504	7494	−1990	1729	2784	1055	32·5	40·3	− 7·8	13·0	20·2	7·2
1885	23	81	6·21	14·10	6228	7210	− 982	2062	3163	1101	40·1	37·8	+ 2·3	15·3	23·0	7·7
1887	10	70	5·15	16·01	5699	6621	− 922	1801	2365	564	34·6	39·6	− 5·0	14·8	19·0	4·2
1897	25	89	12·25	19·08	6246	5476	+ 770	1459	1170	−289	37·3	27·5	+ 9·8	8·8	7·1	−1·7
1900	7	62	4·82	18·45	5871	6085	− 214	1776	1801	25	33·3	34·8	− 1·5	12·3	11·9	−0·4
1911	11	47	6·26	17·63	6721	7565	− 844	1935	2687	752	36·7	40·4	− 3·7	12·5	17·0	4·5
1912	2	80	3·21	23·55	3416	2456	+ 960	942	972	30	18·1	10·7	+ 7·4	4·5	4·2	−0·3
Means	17	93	7·72	17·18	5489	6577	−1088	1686	2186	500	32·5	34·6	− 2·1	11·4	14·8	3·4
							Crops preceded by dry summers and dry winters									
1888	7	56	4·93	11·86	6363	6004	+ 359	1515	1974	459	38·0	33·8	+ 4·2	10·0	12·8	2·8
1898	8	31	6·14	8·46	8943	5397	+3546	2186	3964	1778	38·0	23·7	+14·3	12·0	20·2	8·2
1901	10	58	5·89	13·85	6435	4362	+2073	1627	2062	435	39·6	30·5	+ 9·1	11·7	14·7	3·0
1902	8	37	5·79	10·76	7708	5953	+1755	1853	3729	1876	41·5	33·5	+ 8·0	13·3	22·4	9·1
1903	9	60	5·82	12·28	5244	5077	+ 167	1073	2111	1038	29·7	26·8	+ 2·9	7·6	14·0	6·4
1905	9	44	6·66	11·37	8147	7676	+ 771	3456	2596	−860	38·5	34·2	+ 4·3	18·0	12·9	−5·1
1907	1	41	2·81	16·34	8992	9737	− 745	1715	3094	1379	36·2	34·7	+ 1·5	9·1	14·3	5·2
1909	9	32	7·00	10·29	8164	6794	+1370	1633	2326	693	33·8	26·9	+ 6·9	9·1	12·9	3·8
Means	8	45	5·63	11·90	7537	6375	+1162	1882	2732	850	36·9	30·5	+ 6·4	11·3	15·5	4·2
leans for																

to the character of the preceding summer and winter since the character of the season of growth obviously plays some part; but the latter effect is, as we have seen, largely smoothed out over the number of years.

It is important that agriculturists should realise what great accumulations of nitrate occur in the soil at the end of a dry summer and how complete may be the loss on loams and sands during a mild wet winter. The results on p. 30 show that 50 lbs. or more of nitrogen per acre may easily be lost while the land lies bare between harvest and seed time. Now 50 lbs. of nitrogen per acre is all that is taken out of the soil by a 32 bushel wheat crop. As the prices of nitrogenous manures go on rising it becomes more and more urgent that all waste of nitrogen should be cut down to a minimum; the problem therefore of reducing the winter loss is likely to increase in importance as time goes on.

An obvious method of attacking the problem is by green manuring, and experiments in this direction are being started.

The fact that clay soils retain their nitrates well during winter has already been demonstrated. This appears to be one of the reasons why clays are so suitable for wheat; it is known that wheat requires a supply of nitrates in early life and these are more likely to be present during winter on clay soils than on others.

Discussion of the Results.

On looking over the results the first point that comes out is the rapid accumulation of nitrates in late spring and early summer. During this period the balance of gains over losses is greater than at any other time.

A parallel result has been obtained by Leather at Pusa[1]. During the hot dry season (Oct.—May) relatively little nitrate was formed: after the first heavy rain in June a large increase took place and nitrate was rapidly produced in the first foot of soil: then the action slowed down very considerably and remained slow right on to the end of the wet season. Some of his results, expressed as lbs. of nitrogen per acre, are as follows:—

	Ap 4	May 5	May 25	June 18	June 24	July 21	Aug. 10	Sept. 13
0—6″	·9	2·7	1·2	3·3	·5	·9	·9	·6
6″—12″	·7	·5	·7	18·3	12·8	1·5	·7	·6
12″—18″	·6	·5	·5	·7	6·3	12·0	·7	1·5
18″—24″	·9	·5	·4	·4	1·0	14·0	1·3	1·6

[1] Leather, J. W., "Records of Drainage in India." *Memoirs of the Dept. of Agric. in India*, 1912, II. 101.

C. A. Jensen[1] observed similar phenomena at Bellefourche, S. Dakota. In April only small amounts of nitrate were present in the soil: during the latter half of May and the early part of June a rapid accumulation takes place, but this soon falls off, and at no subsequent period is nitrate formation anything like so quick. Jensen suggests that the organisms have an active growing season of two or three weeks and then their physiological activity is reduced for some cause or other.

On looking over all the evidence the following general rule seems to emerge:—*When a period unfavourable to nitrification comes to an end and more favourable conditions set in, the rate of nitrate accumulation tends to be more rapid in the early part of this new period than later on.*

Several factors have to be taken into account in discussing this rule. In the first instance we know (p. 23) that the accumulation of nitrate ceases after a certain limit is reached, and it probably slackens before this stage. Thus in the dry summer of 1913 the land became so dry that the conditions were unfavourable for nitrification. A wet, warm September followed, when the conditions were distinctly favourable for nitrate production. Yet as a matter of fact little accumulation took place on the unmanured plots, probably because the limit was already reached. Accumulation was more marked on the dunged plots, but again it stopped at a certain point, which appears to be the maximum content for this plot:—

	Dry period		Moist period			Highest amount previously observed
	July 11	Aug. 29	Sept. 3	Sept. 22	Oct. 6	
Hoos fallow, N. as nitrate, parts per million	18	15	17	16	20	18
Moisture in soil, per cent.	8	4	14	12	14	
Broadbalk dunged plot, N. as nitrate, parts per million	7	14	12	17	18	16
Moisture, per cent.	11	10	17	16	18	

Thus these results probably form no real exception to the rule. Again, in interpreting our rapid accumulation in late spring it must be remembered that April, May and June are at Rothamsted somewhat drier than the succeeding months, the average rainfall (60 years) being

[1] Jensen, C. A., "Seasonal nitrification as influenced by crops and tillage." *U.S. Dept. of Agric., Bureau of Plant Industry*, Bull. 173, 1910.

6·48 inches while during the months July, August and September it is 7·56 inches. Further, the winter frosts may cause a certain amount of physical disintegration of the organic matter and render some of it easily assailable by the soil bacteria. Some of the organic matter probably yields ammonia more readily than the rest; while any easily decomposable manures added in spring would obviously give rise to nitrates at an early date. Besides all these possibilities (which affect the decomposable nitrogen compounds) there is another, viz. that the soil bacteria themselves may be more active in the earlier part of the favourable season than later on, or, in our particular case, more active in the spring than in summer and autumn.

Determinations of nitrates do not enable us to decide the question, but investigations made elsewhere seem to indicate that the bacterial activity may be at a maximum in late spring. Löhnis and Sabaschnikoff[1] have shown that the power of decomposing urea and cyanamide shown by the soil at Leipzig is most rapid in spring, then falls off in summer, but rises again in September. The curves obtained resemble my sand curve in Fig. 1. The nitrifying power, ammonifying power, nitrogen-fixing power, and to a less extent the denitrifying power of the soil showed the same type of variation with the season. Müntz and Gaudechon[2] maintain that the nitrifying power of soil is at a maximum in spring. They speak very picturesquely of the awakening of the soil in spring and consider it "une accoutumance, vrai fait d'atavisme" on the part of the soil bacteria. The details of their experiments are open to some criticism[3] but the general result seems to agree with Löhnis' and my own.

It is unnecessary to assume any atavism on the part of the bacteria. The result is entirely in accordance with other work carried out in this Laboratory. It has been shown in various of our papers that the activity of the bacteria of the soil is increased by exposing the soil to conditions unfavourable to active life (e.g. great cold, heat, drought etc.[4]) and then allowing the conditions to become more favourable again. On the other hand the bacterial activity suffers in the long

[1] Löhnis, F. and Sabaschnikoff, *Centr. Bakt. Par.* II. Abt. 1908, **20**, 322—332 and also Löhnis, F., *Vorlesungen über landwirtschaftliche Bakteriologie*, 1913, p. 340.

[2] Müntz, A. and Gaudechon, H., "Le reveil de la Terre," *Compt. Rend.* 1912, **154**, 163—168.

[3] The method was to inoculate soil taken at various dates into soil sterilised at 100° and then to find the amount of nitrification that had taken place. Unfortunately no account was taken of the ammonia produced nor was there any recognition of the special effect of such heated soil in inhibiting the development of the nitrifying organisms.

[4] See Russell and Hutchinson, this *Journal*, 1913, **5**, pp. 167 et seq.

run when the soil is uniformly maintained in favourable conditions[1], it remains at a high level only when unfavourable spells intervene. When soil is partially sterilized the detrimental factor may be completely and permanently thrown out of action and bacterial activity then increases considerably; if the treatment has been insufficiently drastic and the factor is only partially suppressed an increase in activity may still take place, but to a less extent and only temporarily[2].

During the winter months the conditions are unfavourable to the general active life in the soil. We have shown that any unfavourable conditions cause the detrimental factor to suffer more than the bacteria. By spring time we should expect this accumulated differential effect to have become fairly marked so that the bacteria are freer than usual from the detrimental factor. Hence a tendency would be expected for an increase in the rate of nitrate production and (if Löhnis' results are correct) in other bacterial reactions. When later on the detrimental factor recovers, the rate of nitrate production falls off: in the summer therefore a slower rate would be expected, and, as we have seen, it appears to occur.

A similar action would explain the marked rise in the amount of nitrates after the hot dry summer of 1911. The soil became to some extent partially sterilised. This is seen in the clay and the loams; it is not evident in the sand but the moisture content had here run down and remained too low for bacterial action.

Thus these field observations fall into line with our view that two groups of organisms exist in the soil, one engaged in plant food production, and another, which is on the whole detrimental, but is somewhat more affected by adverse conditions.

The next question that needs some discussion is the loss of nitrate during winter. Two reasons are commonly put forward in explanation: denitrification and leaching. In so far as the soil lies waterlogged during winter there is obviously the possibility of the anaerobic conditions necessary for denitrification. The clay and loams investigated were very wet and sticky in winter but not actually waterlogged; the percentage of water in January 1912 was:—

Clay	Loam (Ridgmont)	Loam (Rothamsted)	Sand
29·2	15·5	17·5	9·6

Thus the clay was the wettest, the loams came next, while the sand was driest.

1 Russell and Golding, this *Journal*, 1912, **5**, 27; Russell and Petherbridge, *ibid.* 1912, **5**, 88.

2 Russell and Hutchinson, *loc. cit.* p. 168.

On the other hand percolation is most rapid through the sand and the loams and slowest through the clay, in consequence the amount of leaching is smallest in the clay and greatest in the sand.

These properties enable us to discover whether the main winter losses arise from denitrification or leaching; if from the former we should expect to find them most marked on the wet clay and least on the drier sand; if from the latter they would be most marked on the sand and the loam and least on the clay. Reference to Table I shows that the clay does as a matter of fact suffer less loss of nitrate than the sand and the loams; it starts with a lower quantity in the autumn but ends up with more in the spring.

The most serious loss of nitrate in winter thus appears to arise from leaching and not from denitrification.

Further evidence is obtained from the results of the dunged plots at Rothamsted. In midwinter (Feb. 4th, 1913) the amounts of nitrate in Broadbalk field were :—

Parts per million of soil	Dunged plot (Plot 2)	Unmanured (Plot 3)	N. only (Plot 10)	Complete artificials N. P. K. etc. (Plot 7)
0—9″	10	3	3	6
9—18″	7	4	4	6
lbs. per acre, 0—18″ ..	41	20	18	31
Moisture, per cent.				
0—9″	21·2	15·0	16·6	18·7
9—18″	19·3	16·3	16·1	15·7

While in other fields the amounts were :—

Parts per million	Hoosfield barley plots		Little Hoos rotation plots		Barnfield mangold plots	
	Dunged Plot 7²	Complete artificials 4 A	Dunged plot 5 A *	No dung 1 A	Dunged 1—0	No dung 6—0
0—9′	6	3	5	2	10	4
9—18″	6	3	4	4	7	5
lbs. per acre, 0—18″...	27	16	23	14	41	23
Moisture per cent.						
0—9″	26·2	19·1	18·1	17·0	20·6	15·6
9—18″	18·5	16·8	20·9	16·4	21·9	18·9

* On this plot the dung was applied in the previous October (Oct. 23rd, 1912), on Barnfield it was applied in the preceding April and on Hoosfield in Feb. 1911.

The dunged plots were very considerably wetter than the others; the organic matter caused the water to be held up and thus reduced the amount of percolation. They had lain wet for a long time. All the conditions indeed seemed favourable for denitrification; there were notable quantities of organic matter and a considerable accumulation of water. Yet without exception the dunged plots all contain much more nitrate than the drier plots poorer in organic matter and less favourable to denitrification.

In no case was the land actually waterlogged, so that there was always the possibility of the diffusion of air into the soil; the temperature also was low, although it was above the freezing point. These are common conditions on loams and clays in mild winters and we may conclude that the winter losses of nitrate arise rather from leaching than from denitrification.

The effect of the crop on nitrate production. It has been shown on p. 35 that the amount of nitrate accumulated in cropped land at the end of the season is less than that in fallow land even after allowance has been made for the nitrogen absorbed by the crop. The conditions on a cropped soil are therefore less favourable to nitrate accumulation than those on uncropped land. Two different types of factors may be expected to come into play; negative factors, such as lack of moisture or low temperature on the cropped land, or some positive factor such as a possible direct effect of the growing plant on the nitrate (other than absorption) or on the decomposition processes going on in the soil. In all the samples moisture was determined at the same time as the nitrates, so that we have full data on this point. The figures show less difference than might have been expected between the cropped and the fallow plot, the losses due to the transpiration of the crop being somewhat counterbalanced by the protection against evaporation afforded by the shade of the crop. The following data afford illustration:—

1911......	May 17		June 8		Sept. 13	
	Fallow	Cropped	Fallow	Cropped	Fallow	Cropped
0—9″	14·3	14·8	12·2	10·6	6·2	5·1
9—18″	16·6	16·6	13·7	13·4	12·9	—

per cent. of moisture in the soil

1912

April 19		May 13		June 26		July 22		Sept. 26	
Fallow	Cropped	Fallow	Cropped	Fallow	Cropped	Fallow	Cropped	Fallow	Cropped
10·9	10·8	12·0	9·9	13·0	13·1	12·6	11·7	11·1	10·8
12·2	14·2	15·3	12·5	13·9	15·4	13·7	15·0	14·9	15·2

per cent. of moisture in the soil

Laboratory experiments show that nitrification still goes on when the moisture content is 11 per cent.; less than this amount did not commonly occur in the soil.

Temperature readings were not generally taken, but when they were they showed that the cropped land was cooler than the fallow land in hot weather. It is impossible to say how far these differences in temperature and moisture may have reacted on the rate of nitrate production, and whether they are sufficient to account for the whole of the difference observed.

This depressing effect of the crop on the rate of nitrate accumulation has been observed before. Eight years ago Warington[1] showed that the amount of nitrate in the drainage water from Broadbalk field was considerably less than was expected from the manure supplied and the crop reaped. The result is not wholly experimental, for it involves certain assumptions as to the amount of water draining away for which no direct evidence could be obtained; nevertheless as they were drawn up by Sir Henry Gilbert they deserve very serious consideration. Warington thought that denitrification might account for some of the discrepancy but not for all, as it could hardly be supposed to act in dry summer weather: he further suggested that the nitrate might be taken up by the plant and then somehow lost before harvest. More recently Lyon and Bizzell[2] found *more* nitrate on land cropped with maize (after allowing for the nitrogen present in the crop) than on fallow land of similar previous history, and concluded that the growing maize plant in some way stimulated nitrification. During the latter part of the life of the plant less nitrate was found in the cropped than in the fallow land, and the further conclusion is drawn that nitrification is inhibited

[1] *Trans. Highland Agric. Soc.* 1905, **17**, pp. 175 et seq.

[2] Lyon, T. Lyttleton and Bizzell, James A., *Journal of the Franklin Institute*, Jan.—Feb. 1911, "The Relation of certain Non-leguminous Plants to the Nitrate Content of Soils." (All their figures are quoted as NO_3 but for convenience of comparison I have also reduced them to N.)

by the conditions accompanying the decreasing activities of the roots. On the other hand where oats and potatoes were grown the nitrates were never so high in the cropped as in the uncropped land, again, apparently, after allowing for what has been absorbed by the crop. The following amounts of nitrogen as nitrate occurred in parts per million of soils :—

1908	Fallow land		Land carrying maize		1909	Fallow land		Land carrying oats	
	as NO_3	as N.	as NO_3	as N.		as NO_3	as N.	as NO_3	as N.
May 19	21·9	4·9	17·5	3·9	April 22	84·0	19·0	48·1	10·9
June 22	48·1	10·9	41·3	9·3	June 24	55·7	12·6	11·1	2·5
July 6	64·1	14·5	62·8	14·2	July 12	55·8	12·5	4·6	1·0
July 27	186·8	42·1	191·2	43·2	Aug. 7	81·6	18·4	3·7	0·8
Aug. 10	178·6	40·3	165·3	37·3					

It is interesting to observe that the figures are generally of the same order as ours excepting only in July and August 1908.

I have never observed any increase in nitrate on cropped land such as is recorded in the maize experiments of Lyon and Bizzell ; my results with wheat and barley have always shown a decrease, like theirs with oats. Leather's experiments[1] also show a decrease. The nitrate in the drainage water from the fallow gauges at Pusa contained respectively 261·5 and 209·6 lbs. per acre during the period 1907—9, while that in the drainage water and crops of the gauges cropped with grass accounted only for 128·4 and 115·6 lbs. per acre over the same period. The final rainfall before the account was made up was so heavy as to deplete the gauges of nitrate, so that no error arises through the retention of nitrate in the soil.

Dehérain's experiments[2] made at Grignon, near Paris, between 1892 and 1897 also showed much more nitrate coming from the fallow lysimeters than from those covered with crops even after allowing for what was absorbed by the crop. In this case, however, it is uncertain how much nitrate was left in the soil, the rainfall probably being insufficient to wash it all out.

It seems to be an established fact that *less nitrate accumulates, and apparently less nitrate is produced, on cropped land than on fallow land,*

[1] "Records of drainage in India," J. Walter Leather. *Memoirs of the Dept. of Agric. in India*, Chemical Series, 1912, II, 63—140.

[2] *Traité de chimie Agricole*, M. Dehérain, pp. 584—599.

even after allowing for the nitrate absorbed by the crop. This result has been obtained under such widely different climatic conditions as prevail at Rothamsted in England, at Pusa in India, at Ithaca in New York State, and apparently at Grignon near Paris[1]. Although the actual experimental figures refer only to the accumulation of nitrate we are probably justified in supposing that they indicate a diminished *production* of nitrate in cropped land, otherwise we have to assume some destructive process at work in the cropped soil that does not go on in the fallow soil, an assumption for which there is no evidence at all. The wide range of climatic conditions under which the result is obtained seems to preclude any assumption that the diminished production is due to the effect of the crop on the temperature or moisture content of the soil. There appears to remain only the possibility that the growing plant has a direct effect on the decomposition processes going on in the soil. Unfortunately field experiments alone do not enable us to decide this question; there is, however, sufficient indication of a direct effect to justify a systematic investigation of the problem.

On the Determination of Nitrates in Soils.

The method adopted in the Rothamsted laboratory was originally devised by Warington and consists in extracting the soil with water, reducing the solution with a zinc-copper couple[2] and estimating the ammonia in the usual way. It is both simple and accurate, and has now become part of our regular laboratory routine.

The details are as follows: The soil is brought down as rapidly as can be from the field; it is sampled, lots of 200 grams are weighed, put to dry[3] in a chamber at 38°—40° and weighed again when dry. Then

[1] A similar result seems to have been obtained by B. Welbel in the lysimeter experiments at Ploty, Podolie, Russia. In the French summary of its 11th Report (for 1905) he states:—"Les cultures en vases montrent encore que les plantes fourragères possédent une influence individuelle sur l'énergie des procès de nitrification: ainsi l'influence de l'esparcette est supérieure à celle de la luzerne." Unfortunately the details are given only in the Russian text.

[2] Williams' method, *Trans. Chem. Soc.*, 1881, **39**, 100.

[3] It is not necessary to dry light soils and they can be extracted straight away with water. Heavy soils, however, do not usually allow sufficient percolation to admit of extraction until they have first been dried as directed; our Rothamsted soils, for example, have to be treated in this way as a rule. We are not prepared to say that the drying is without effect on the nitrate content, but we find that it greatly facilitates extraction and it leads to higher and more uniform results than are obtained otherwise. As a precaution, however, all the soils throughout a given investigation are invariably treated alike, and either all dried, or, if they are sufficiently pervious, all extracted in the fresh state.

the soil is roughly pounded in a mortar, put on to a Buchner funnel and extracted with 600—800 c.c. of distilled water. To the extract is added a small quantity of ignited magnesia (about 0·05 gram); it is then concentrated in a Jena glass beaker to about 100 c.c., transferred to an 8 oz. bottle $5\frac{1}{2}$ ins. high to the shoulder and 2 ins. diameter, and acidified with acetic acid. Some care is needed here to see that all the magnesia is dissolved; part may remain unaltered owing to the narrowness of the bottle even though the solution gives at first an acid reaction.

Two pieces of the zinc-copper couple are then added. These are prepared from strips of zinc foil 4 inches long and 2 inches wide bent into half-circles round a cylindrical piece of wood. The strips are immersed for a few minutes in caustic soda solution, then washed under the tap and immersed in dilute sulphuric acid, again washed in water and transferred to a 2 per cent. solution of copper sulphate. In a few seconds a dark coating of copper is deposited; the strip is taken out, at once placed in distilled water for a few seconds and then dropped in to the acidified soil extract. The bottle is now corked and placed in an incubator kept at 25—30°[1] for 2 days. Reduction is by this time complete and the ammonia may be distilled off and estimated by titration, or, in the case of small quantities, by nesslerisation.

The following data show that the method gives satisfactory results. A known quantity of sodium nitrate was added to soil, the total amount of nitrate was then determined; the results show that the amount found corresponds closely with what was expected:—

	N. as nitrate originally present	N. as nitrate added	N. as nitrate expected	N. as nitrate found
Soil A	24	11	35	35
	24	5	29	29
Soil B	12	10	22	22

Parts per million of dry soil

It does not appear that the determination is complicated by the presence of other nitrogenous organic substances that might be expected

[1] A worn out steam oven does very well for this; if the space between the walls is filled with water it is not difficult to keep the temperature within proper limits.

in arable soils. Urea, however, is hydrolysed to some extent and causes the results to come out rather too high :—

Compound added to soil	N. in added compound	N. as nitrate originally present in soil	N. as nitrate found	Error caused by added compound
Asparagine	5	17	18	1
,,	7·5	29	29	none
,,	10	12	12	none
,,	15	24	25	1
Betain	5	17	18	1
,,	10	12	13	1
Peptone	5	17	18	1
,,	10	12	13	1
Urea	5	17	19	2
,,	10	12	15	3

Parts per million of dry soil

Urea is so easily decomposed in the soil that it is not likely to give rise to difficulties in practice, while the small error introduced by the other substances is, as we shall see, less than the error of sampling.

Pasture soils and very heavily manured soils often give a dark coloured extract containing nitrogenous compounds of unknown constitution, and in such cases it is probably not safe to regard the figures as representing the nitrates only, but to give some wider designation as has already been done in some of our papers[1].

Various reducing substances appear to affect the determination and the method does not always give reliable results for pot experiment work (e.g. where calcium sulphide has been added to the soil). In experiments of this kind it is necessary to ascertain whether the method holds before embarking in a series of determinations.

The variation in the field. A fair amount of uniformity exists in the nitrate content of a plot which has been uniformly treated and the differences between the various mixed samples do not generally amount to more than about 2 parts per million. Larger differences, however, occur when dung has recently been applied owing to the difficulty of getting a regular distribution. At least three cores have to be taken even on a tolerably uniform plot.

The following results show the kind of variation that is obtained among samples taken from the same plot when single cores only are taken :—

[1] E.g. this *Journal*, 1912, **5**, 27.

Sample	Broadbalk orchard, 1909						Barnfield, April 18, 1913				Harpenden Field, May 14, 1913	
	April 6		July 6		Oct. 28		Unmanured (8—0)		Dunged*			
	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″
1	4	4	6	4	5·5	6	4	4	8	6	7	9·5
2	4	4	7	7	5·5	9	3	4	17	4·5	13	9
3	—	—	—	—	—	—	2·5	2·5	—	7	10	8·5

* Dung applied March 28, 1913.

But when mixtures of three cores are taken the results are more uniform so long as the soil is dried before analysis:—

	Soil dried before analysis				Soil analysed fresh from field			
	Hoos fallow		Hoos dunged plot		Hoos fallow		Hoos dunged plot	
	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″	0—9″	9—18″
1st three cores	7	7	9	6	7	8	6	6
2nd ,, ,,	9	5	10	4	9	3	5	3

Parts per million

	0—18″	0—18″	0—18″	0—18″
1st three cores	37	34	40	28
2nd ,, ,,	37	31	32	18

lbs. per acre

Summary and Conclusions.

The amount of nitrate in the soil of arable land fluctuates regularly but in these experiments it rarely exceeded the following values:—

	Per million	Per cent.	lbs. per acre 0—18″
Sand	6	·0006	28
Loam	23	·0023	115

(excepting on heavily dunged land, when it rose to 37 parts per million)

Clay	14	·0014	60

In almost all the soils examined the accumulation of nitrate took place most rapidly in late spring or early summer. After this there was usually little if any gain and very frequently a loss. In the hot

dry autumn of 1911, however, the accumulation continued in some of the soils right on till September.

During the winter loss of nitrate takes place. This was more marked in the wet winter of 1911—12 than in the drier winter of 1908—09.

The fluctuations in nitrate content are more marked on loams than on clays or sands. Clays lose less of their nitrates in winter, but on the other hand they accumulate smaller amounts in June and July. Sands lose much of their nitrates in winter and do not accumulate very large amounts in summer. It appears that the main loss in winter is due to leaching and not to denitrification.

On comparing the nitrate content of cropped and fallow land it is found that during late summer and early autumn the fallow land is the richer even after allowing for the nitrate taken up by the crop. Indeed no evidence could be obtained that any nitrate was produced in the soil during the time of active crop growth, although nitrate accumulation was taking place on adjacent fallow land. The question arises whether the growth of a crop exerts any effect on the rate of nitrate production in the soil. The data to hand do not enable us definitely to settle this point.

The rapid rise in nitrate content in spring does not usually set in immediately the warm weather begins; there is a longer or shorter lag. There are indications of greater bacterial activity in early summer than later on, a phenomenon readily explicable on our view that the soil population is complex and includes organisms which are detrimental to the decomposition of bacteria but which are on the whole more readily put out of action.

The supply of nitrate to the plant is known to be a factor of prime importance in plant growth. Similarly it is found that the factors which determine the accumulation of nitrates in the soil also play a great part in determining the amount of crop production. Thus heavy winter rainfall, which washes out nitrates, tends to reduce crop growth: on the other hand hot dry summers succeeded by dry winters are favourable to nitrate accumulation and therefore to crop growth.

ESTIMATION OF THE SURFACE OF SOILS.

By J. A. HANLEY.

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DURING the last few years a good deal has been heard of the estimation of colloids in soils by means of dye solutions.

The method is described by H. E. Ashley[1] and later by König, Hasenbäumer, and Hassler[2].

These workers used colorimetric methods of estimating the dye absorbed. R. van der Leeden and F. Schneider[3] used a titration method.

B. Sjollema[4] tried several dye solutions and finally adopted methyl violet dissolved in water as being the most general dye for both basic and acidic constituents of the soil; other dyes such as naphthol yellow, alizarin, and congo red were found to be selective.

König, Hasenbäumer, and Hassler used methyl violet and methyl green but recommend the former as its colour undergoes only a very slight change after being in contact with the soil.

These workers shook up samples of soil in cylinders with solutions of methyl violet and compared the resulting solutions with standard dye solutions of strengths varying from 0·3 mg. to 40 mgs. per 100 c.c.s.

From work described by other workers, particularly Dreaper and Davis[5], it appears that the dye colours not only colloidal particles but particles of all sizes. Dreaper and Davis used fine sand washed and cleaned with HCl. Their method was to run dilute dye solution from a burette through vertical tubes packed with sand, the end-point being

[1] *U.S. Geol. Survey Bull.* 1909, 388.
[2] *Landwirtsch. Versuchs Stat.* 1911, **75**, 377.
[3] *Inter. Mitt. für Bodenkunde*, II. 81.
[4] *Jour. für Landwirtsch.* 1905, 67.
[5] Inter. Congress of Applied Chemistry, 1909, Sect. IV B.

reached when the first coloured drop passed through; this gave a very sharp end-point.

In the work to be described here three soils were used, of which the mechanical analyses were known, and the first method adopted for dyeing them was that used by Dreaper and Davis with methyl violet as the dye.

This method although rapid for sands was found to be unsatisfactory for soils containing any clay, because, even by using a filter pump, it was difficult to get the solution to run through, and also because the soil dyed very unevenly, the colour being much denser at the top of the tube than the bottom when the filtrate first became coloured; moreover the dye solution tended to run in channels amongst the coarser particles.

This led to the adoption of König, Hasenbäumer, and Hassler's method. Glass tube-bottles of about 150 c.c.s capacity with ground-in stoppers were used. Five gms. of air-dried soil were shaken with 100 c.c.s of methyl violet solution and laid on their sides for 24 hours with occasional shaking and then stood upright for another 24 hours to allow the soil to settle. Part of the top solution was then pipetted off, diluted suitably, and the dye estimated colorimetrically by means of Nessler's tubes and standard dye solution. König used different strengths of dye solution for different soils, viz. 1 gm. per litre for sandy soils, 2 gms. per litre for loams and 3 gms. per litre for clays.

If however the weakest solution is used for a clay, the soil does not take up so much dye and yet leaves some in solution. Again if the strongest solution be used for sands the soil takes out far more dye than it will from a weak solution.

It was therefore decided to compare the dye taken out by the soils chosen, from solutions of varying strengths.

The three soils chosen were (1) a sandy loam with 3 °/₀ clay, (2) a loam with 8 °/₀ clay, (3) a Rothamsted soil (clay loam) with 20 °/₀ clay.

The dye solutions were of twelve different strengths varying by 0·25 gm. per litre from 0·25 gm. per litre to 3 gms. per litre and curves were drawn representing the amount of dye absorbed per 100 gms. of soil against the amount of dye left in the solution. The three curves are shown in the accompanying figure. In all cases the dye absorbed increases rapidly with increasing strength of dye solution up to a certain point when the soil appears to become almost saturated.

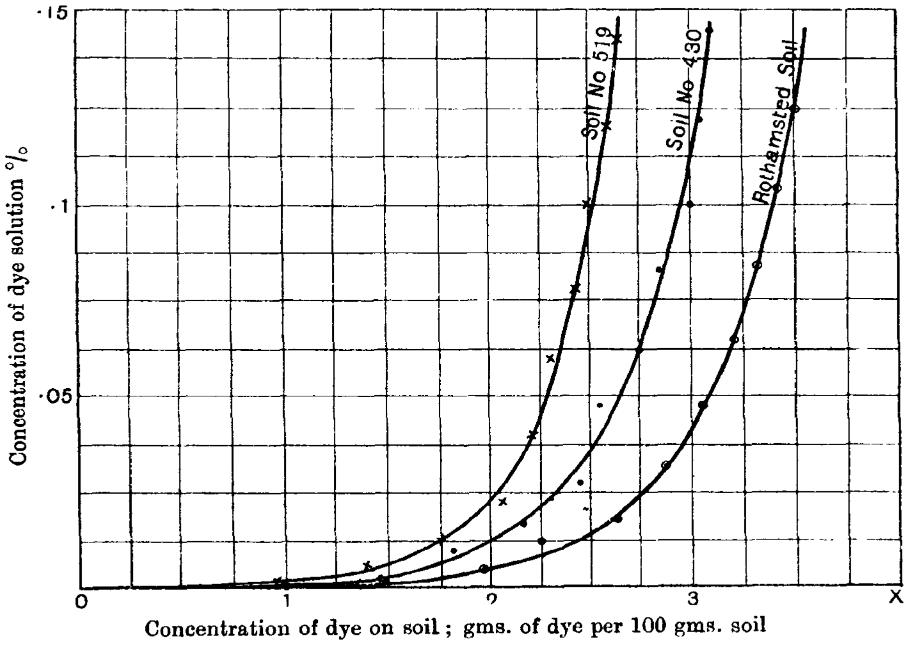
It is obvious from these curves that if dye solution of one strength be used the three figures obtained cannot be comparable.

The results obtained for the three soils and from which the curves were plotted are given in Table I.

TABLE I.

Dye solution used, gms. per litre	Soil No. 519, Dye per 100 gms. soil: Absorbed	Soil No. 519, Dye per 100 gms. soil: Left	Soil No. 430, Dye per 100 gms. soil: Absorbed	Soil No. 430, Dye per 100 gms. soil: Left	Rothamsted soil, Dye per 100 gms. soil: Absorbed	Rothamsted soil, Dye per 100 gms. soil: Left
0·25	·50	—	·50	—	·50	—
0·50	·97	·03	·98	·02	·99	·01
0·75	1·38	·12	1·46	·04	1·45	·05
1·00	1·75	·25	1·80	·20	1·90	·10
1·25	2·05	·45	2·15	·35	2·25	·25
1·50	2·20	·80	2·45	·55	2·63	·37
1·75	2·30	1·20	2·55	·95	2·86	·64
2·00	2·44	1·56	2·75	1·25	3·05	·95
2·25	2·50	2·00	2·84	1·66	3·20	1·30
2·50	2·60	2·40	3·00	2·00	3·32	1·68
2·75	2·65	2·85	3·05	2·45	3·42	2·08
3·00	—	—	3·10	2·90	3·50	2·50

Curves showing points of equilibrium of dyed soil with dye solution.



To obtain relative values indicating the active surfaces of different soils it is necessary that each soil is brought into equilibrium with a solution of the same strength. For example, for the three soils used the only points by which the soil surfaces can be compared lie on lines parallel with the OX (dye absorbed) axis.

If we use a dye solution of say 1 gm. per litre for all three soils, each dyed soil is in equilibrium with a different solution at the end of the experiment and more dye must be added to the one with 20 °/ₒ clay before the final condition is comparable with that of the soil with 3 °/ₒ clay.

It is necessary to use then not the same strength of dye solution throughout, but such a strength for each soil as will leave it when dyed in equilibrium with the same dye solution.

It is obvious that if the method is to be of use the relative values for different soils ought to be the same whatever the concentration of the final solution.

In Table II the values are taken from the curves for dye absorbed by each soil when in equilibrium with solutions of strengths varying by 0·0125 gm. per 100 c.c.s from 0·0125 to 0·125 gms. per 100 c.c.s.

TABLE II.

Equi-librium solution °/ₒ	Readings on horizontal axis of curves			Readings reduced to Rothamsted = 1		
	519	430	Rotham-sted	519	430	Rotham-sted
·0125	7·0	8·0	9·6	·73	·83	1
·025	8·1	9·3	11·0	·74	·85	1
·0375	8·7	10·1	11·8	·74	·86	1
·05	9·1	10·6	12·3	·74	·86	1
·0625	9·4	11·0	12·8	·74	·86	1
·075	9·7	11·3	13·1	·74	·86	1
·0875	9·9	11·6	13·4	·74	·87	1
·1	10·1	11·8	13·6	·74	·87	1
·1125	10·25	12·0	13·8	·74	·87	1
·125	10·4	12·2	13·9	·75	·88	1

In the second part of Table II the same results are expressed in terms of the Rothamsted soil (taken as 1).

It will be seen that the values on the lower parts of the curve where the dye solutions are weak vary considerably, but from ·0375 °/ₒ to ·1125 °/ₒ they are constant.

The experimental error is also greatest at the extremities of the curve, in the one case the remaining solutions in which the dye is estimated are very weak and in the other case they are concentrated and need considerable dilution.

In the actual experimental work then the equilibrium solution chosen should be one between ·0375 °/$_{\circ}$ and ·1125 °/$_{\circ}$; the most convenient is ·05 °/$_{\circ}$, little dilution is required and the figure is a convenient one for the resulting calculation.

The method adopted for obtaining the requisite equilibrium mixture was to use four solutions for each soil, viz.:

For sands	For loams	For clays
·075 °/$_{\circ}$	·1 °/$_{\circ}$	·15 °/$_{\circ}$
·1	·15	·2
·125	·2	·25
·15	·25	·3

The standard equilibrium solution of ·05 °/$_{\circ}$ is treated in the same way as those containing soil. After the 48 hours each solution is diluted 1 in 10, i.e. 10 c.c.s of the top solution is pipetted off and made up to 100 c.c.s, and these solutions are compared with the standard roughly by holding against the light.

The two nearest the standard solution are compared with it accurately by means of Nessler's tubes, and the dye absorbed in equilibrium with a solution containing ·05 °/$_{\circ}$ can easily be deduced.

It is intended to continue this work in order to see whether other factors, such as temperature and original amount of water in the soil, influence the percentage of dye taken up by soils; and also to compare the results with values obtained for the 'hygroscopic water' content of soils. In case of some definite relationship existing between the two, the dye absorption method, which is fairly rapid and easy of manipulation, would give a good deal of valuable information regarding the relations between surface and the water content of soils, information that is difficult to obtain at present on account of the very unreliable methods available for its determination.

THE HUMUS OF ACID AND ALKALINE PEATS.

By J. A. HANLEY.

(The University of Munich, formerly at the Rothamsted Experimental Station.)

THE necessity for a method of comparing and, if possible, distinguishing chemically between the different classes of peat, and especially between alkaline or normal peats and acid peats, was suggested by a collection of soils received at the Rothamsted Laboratory.

These soils were collected from widely distributed areas throughout Great Britain and were not as a rule under cultivation.

A great many of them were acid, infertile peats from bogs and moors in England and Scotland, which *in situ* carried nothing but a useless vegetation specially adapted to their acid nature; whilst others were alkaline or normal peats from districts like the Fens, very fertile and yielding useful crops when in cultivation.

These peats contained from 30 °/₀ to 70 °/₀ of organic matter (loss on ignition) and it is obvious that this was the main factor determining the characteristics of each particular soil.

This organic matter differed greatly both physically and chemically in the two classes of peat and had undoubtedly been formed under very different conditions and from a different type of vegetation (probably also by different organisms).

If therefore an attempt were to be made to bring the acid peats into a state of fertility, the first consideration, after rectifying drainage etc., would be the nature of this dominating constituent of the soil which would become the main source of plant food, particularly of nitrogen.

If air-dried samples of an alkaline fen peat and an acid moor peat are treated with dilute ammonia, the extract from the acid peat is much

darker in colour and contains more dissolved organic matter than does the alkaline peat extract.

Conversely, if they are treated with acid the alkaline peat yields the darker extract.

It is generally considered that the 'humus' is formed by the breaking down of fresh vegetation in the soil into simpler forms of nitrogen compounds to which the term 'humic acid' is applied. In a normal soil containing sufficient lime this mixture of acids is neutralised by the lime with the formation of 'calcium humate,' whereas in acid soils devoid of lime the 'humic acid' retains its acid form.

In ordinary soil analysis, when 'humus' is estimated by standard methods, 'humic acid' is regarded as the form of humus dissolved out by the ammonia and therefore the 'humates' are first broken down by treatment with dilute HCl.

This led to a suggestion by Mr A. D. Hall that the 'humus' should be determined in this series of soils by solution in 4 °/₀ ammonia before and after treatment with dilute HCl. Typical acid, alkaline, and neutral soils were treated in this way.

10 gms. of soil were treated for 24 hours with 200 c.c.s of N/5 HCl, well washed, filtered, and then dried at 100° C. to get rid of the last trace of HCl. The soil was then transferred to a litre bottle and shaken with 500 c.c.s of 4 °/₀ NH_3. The bottles were laid on their sides with occasional shaking for 24 hours and then stood upright for 12 hours to allow the soil to settle. 200 c.c.s was then filtered off (or in the case of clayey soils, which become very deflocculated in the ammonia, the top 200 c.c.s was carefully decanted off) and evaporated to dryness in a platinum dish, dried at 100° and weighed; ignited and weighed again. The loss in weight being taken as 'humus.'

In the case of estimation of humus without acid treatment the dried soil is put into the bottle direct and ammonia added.

Care should be taken in evaporating the ammonia extract as a slight rise in temperature above 100° results in considerable loss of organic matter.

The results of this analysis for 35 of these soils are shown in Table I.

The humus results are expressed as percentages on the air-dried soil and in the last column the ratio between the two humus determinations is given.

If this method holds good as a method for determining acidity, the soil with the highest ratio should be the most acid, i.e. it should have the largest proportion of 'humus' as 'humic acid.'

TABLE I.

Soil No.	Re-action to litmus	% $CaCO_3$	Loss on ignition	Humus 1 (after acid treat-ment)	Humus 2 (no acid treat-ment)	Ratio $\frac{\text{humus 2}}{\text{humus 1}}$	Locality
				% on air-dried peat			
514	acid	nil	16·08	3·450	3·525	1·023	Birch Wood, Scotland
486	acid	nil	26·99	13·020	13·190	1·014	——
442	acid	nil	74·20	6·450	6·250	·970	Norfolk Broads
530	acid	nil	26·69	8·325	7·750	·930	Derbyshire
502	acid	nil	14·26	4·600	3·675	·800	London Clay
478	?	—	35·26	2·375	1·848	·779	Ant Valley, Broads, Norf.
482	acid	nil	67·00	6·500	4·975	·766	,, ,,
460	?	—	70·41	9·375	6·580	·703	,, ,,
475	acid	nil	24·46	6·430	4·500	·700	,, ,,
492	acid	nil	15·61	4·750	3·250	·685	London Clay
450	acid	nil	12·75	6·722	4·450	·662	Ant Valley, Broads, Norf.
441	acid	nil	67·05	10·590	6·950	·655	,, ,,
459	alk.	—	40·23	3·050	1·924	·631	,, ,,
451	alk.	—	56·15	10·940	6·650	·608	,, ,,
458	alk.	—	70·74	5·275	3·125	·593	,, ,,
471	acid	—	75·80	7·125	4·025	·565	,, ,,
472	alk.	3·85	11·24	1·800	1·000	·556	,, ,,
455	alk.	—	72·92	6·350	3·450	·543	,, ,,
449	?	—	23·07	4·575	2·475	·541	,, ,,
443	acid	—	63·84	7·070	3·700	·524	,, ,,
456	alk.	—	55·57	12·440	6·500	·522	,, ,,
462	acid	—	74·27	10·970	5·700	·520	,, ,,
446	?	—	69·65	7·900	4·050	·513	,, ,,
466	alk.	—	41·96	3·250	1·650	·508	,, ,,
470	acid	nil	2·84	2·175	1·074	·494	,, ,,
453	acid	nil	16·52	3·175	1·525	·481	,, ,,
454	?	—	60·60	6·950	3·150	·453	,, ,,
484	alk.	1·7	8·00	2·400	1·024	·427	,, ,,
477	?	0·13	16·86	4·675	1·898	·407	,, ,,
483	acid	—	51·33	14·590	5·525	·379	,, ,,
444	?	—	64·30	10·120	3·475	·343	,, ,,
447	acid	—	56·27	6·650	2·175	·327	,, ,,
445	?	—	67·49	11·900	3·425	·288	,, ,,
500	alk.	0·3	7·36	3·775	1·075	·285	London Clay
421	v. alk.	—	74·60	11·680	2·925	·250	Adventurer's Fen, Camb.

The soils have been tabulated in the order of this ratio and it will be noticed that many alkaline soils occur high in the series. It appears therefore that soils may be alkaline in reaction and yet contain humus soluble in ammonia.

The methods employed at this stage of the work for indicating whether a soil was acid or alkaline were two. (1) The litmus test: a red and a blue litmus paper were placed side by side in a porcelain dish containing one or two c.c.s of distilled water and a small sample of soil was dropped on to the two papers and pressed down with a glass rod. The acidity or alkalinity was then expressed roughly as v. acid, acid,

sl. acid, neut. etc. according to the readiness with which the paper was coloured.

(2) By estimation of $CaCO_3$: the method described by Amos[1] and modified by Marr[2] was used, great care being taken in the case of soils rich in organic matter to use very dilute acid and reduced pressure.

In all about 200 soils were compared by these methods and in no case did a soil giving an acid reaction towards litmus evolve any carbon dioxide and *vice versa* all alkaline soils gave indications of $CaCO_3$.

The results of these tests for the typical soils used are given in the 2nd and 3rd columns of Table I.

This preliminary portion of the work leads to the following conclusions.

(1) In every soil, containing an appreciable quantity of organic matter, part of the humus is soluble in ammonia without previous acid treatment.

(2) Soils containing no $CaCO_3$ give an acid reaction to litmus and no acid soil contains $CaCO_3$. Indeed, the hitherto rather vague term 'acid soil' is gradually assuming a more definite meaning, viz. a soil deficient in carbonates.

In view of the conflicting results obtained with 4 °/o ammonia it was decided to study in more detail the 'humus' of a few typical peats drawn from quite different localities.

The five peats chosen were:

No. 850. Peat from Rothamsted grass plot 11 (1), an example of an acid peat produced from quite normal vegetation by continuous application of acid manures.

This plot receives in addition to excess of mineral manures a large excess of ammonium sulphate.

No. 851. A typical alkaline fen peat from Cambridge Fens, very black in colour and containing a considerable quantity of ferrous sulphide, as is the case with many of these fen peats. Very little unchanged vegetable material was noticeable in this peat.

No. 852. An Irish bog peat reddish in colour and containing a large proportion of fresh fibres etc. This peat is very acid and infertile.

No. 853. A fertile Irish peat closely resembling No. 851 in appearance with no fresh vegetable matter and very little or no sulphide.

No. 854. A typical acid moorland peat from the Millstone Grit, near Halifax, Yorks, probably a Sphagnum peat.

[1] This *Journal*, 1905, 322. [2] *Ibid.* 1909, 155.

These peats were first treated in the same way as the others by solution in 4 °/₀ ammonia with and without previous acid treatment.

As before, large proportions of humus were dissolved out without previous acid treatment, the most abnormal case being the neutral Irish peat 853 which yielded 15·58 °/₀ in this manner. The ratio was also as high as 0·65, greater than that of any alkaline peat examined earlier.

TABLE II.

Soil No.	Reaction to litmus	Loss on ignition	4 °/₀ amm. after acid	4 °/₀ amm. no acid	Humus ratio $\frac{\text{no acid}}{\text{acid}}$
850	acid	48·75	10·07	12·24	1·22
851	alk.	57·14	21·73	2·43	·11
852	acid	80·42	12·07	9·83	·81
853	neut.	59·80	24·10	15·58	·65
854	acid	87·95	7·10	9·53	1·34

Nitrogen was estimated in both these ammonia extracts and compared with the total nitrogen in the peats and with the 'humus.'

The ammonia was got rid of by distilling 150 c.c.s of the extract with magnesia at 40° C. under reduced pressure, until the whole of the liquid had passed over and the contents of the flask were dry. These were washed out with strong sulphuric acid and Kjeldahled in the usual way. The results are given in Table III.

TABLE III.

Soil No.	Total Nitrogen °/₀ in air-dried peat	A, N in humus after acid			B, N in humus without acid			Ratio of N (B) to N (A)
		°/₀ in air-dried peat	°/₀ in humus	°/₀ in total N	°/₀ in air-dried peat	°/₀ in humus	°/₀ in total N	
850	1·418	·745	7·40	52·6	·896	7·32	63·2	1·20
851	1·710	1·014	4·67	59·3	·114	4·68	6·7	·11
852	1·192	·803	6·66	67·3	·577	5·87	48·3	·72
853	1·269	1·110	4·62	87·4	·852	5·47	67·1	77
854	0·885	·547	7·70	61·8	·554	5·82	62·7	1·01

These results are indefinite but might be taken to indicate: (1) the ammonia dissolves out substances of very nearly the same N content

whether the peat is or is not previously treated with acid and (2) the composition of the humus as regards N varies only slightly in different peats.

Obviously some of the nitrogenous matter dissolved by 4 % ammonia alone may be dissolved out by the HCl and be therefore lost in the determination of humus after acid treatment. The nitrogen thus lost was estimated and the results are given in Table IV where the N is expressed as percentage of air-dried peat and of total N.

TABLE IV.

Soil No.	N dissolved by N/5 HCl		Total N in peat	Total N extracted by acid and subsequent ammonia	Ratio of N extracted to total N
	% in air-dried peat	% of total N			
850	0·140	9·88	1·418	·885	62·4
851	0·056	3·28	1·710	1·070	62·7
852	0·100	8·40	1·192	·903	75·8
853	0·030	2·36	1·269	1·140	89·8
854	0·030	3·39	·885	·577	65·2

In the second part of the table the nitrogen dissolved out by N/5 HCl is added to that extracted by 4 % ammonia after acid treatment and the total nitrogen extracted is given. The ratio of the extracted N to the total N is given in the last column.

The percentage of N extracted by N/5 HCl from 850 is abnormally high—practically 10 % of the total N, and again when the N extracted by different methods is calculated as percentage of that organic matter the results obtained for 850 are far in excess of either of the other acid peats. These high results may be accounted for by the large amount of nitrogenous manures given each year, some of which may be present in the peat in an easily soluble form.

To eliminate any error due to loss of nitrogen, originally in the peat, but which may have been converted during the process of humus estimation into forms losing ammonia on distillation with magnesia, estimations were made using caustic soda instead of ammonia. The soda was made up to a strength equivalent to 4 % ammonia.

It was found to be impossible to estimate the total organic matter dissolved out by soda, but the solution was neutralised with sulphuric acid and Kjeldahled in the usual way. The results are given in Table V.

TABLE V.

Soil No.	N from NaOH extract after acid treatment			N from NaOH extract without acid treatment		Ratio $\frac{\text{no acid}}{\text{acid}}$
	°/₀ in air-dried peat	°/₀ of total N	Total N extracted by soda + acid	°/₀ in air-dried peat	°/₀ of total N	
850	·935	65·8	1·075	1·016	71·7	1·09
851	·935	54·7	·991	·444	26·0	·48
852	·533	44·7	·633	·514	43·2	·96
853	·851	67·0	·881	·483	38·1	·57
854	·326	36·9	·356	·388	43·8	1·19

After acid treatment the alkaline peats yield the highest percentage of nitrogen soluble in alkali, with the exception of the abnormal 850. The soda gives lower results than the ammonia, so that the soda dissolves either different compounds or different amounts of the same compounds. The ratios of nitrogen obtained without and with previous acid treatment appear to be far more significant than such results obtained with 4 °/₀ ammonia. The two alkaline peats give correspondingly low results, whilst the acid peats all give high ratios. The soda ratios correspond with the 'humus' ratios but not with the 'N in humus' ratios.

Soil 853 appears to be abnormal with ammonia but normal with soda, that is the soda and ammonia dissolve different substances and very different amounts of nitrogen; 87·4 °/₀ and 67·1 °/₀ of the total nitrogen in the case of ammonia and only 67 °/₀ and 38·1 °/₀ in the case of soda.

Another comparison of the five peats was made by an estimation of the nitrogen formed by hydrolysis of proteins and similar compounds in the peat.

The method adopted was the Osborne-Harris modification of Hausmann's method for determining nitrogen in proteins.

10 gms. of peat was boiled with 100 c.c.s of 20 °/₀ HCl for 10 hours. The whole was transferred to a distilling flask and evaporated under diminished pressure at 40° C. to get rid of the HCl and then distilled under the same conditions with MgO.

The results (Table VI) show that only the alkaline peats gave any ammonia and these quantities were small for the very alkaline peat 851. The method would therefore be of little use for distinguishing soils on the border-line of acidity and neutrality.

So far the only acid property of the 'humic acid' of peats dealt with has been solution in alkalies, and since different alkalies appear to

TABLE VI.

Soil No.	850	851	852	853	854
N (estimated as NH_3) after hydrolysis. °/₀ on air-dried peat	nil	·092	nil	·014	nil

react differently towards these nitrogen compounds, it was decided to use another acid property of 'humic acid' described by Tacke and Süchting[1], i.e. their power of inverting sucrose.

The method has been used for comparing quantitatively the acidities of soils other than peats and will be described in detail at the end of the paper. Here only sufficient detail will be given for continuing the comparison between the five peats.

It was found that if a solution of sucrose is boiled for some time with peats and the amount of glucose produced is estimated, the percentage of sugar inverted varies considerably with the type of peat.

In the results given the amount of inversion is expressed as weight of Cu_2O obtained, this being directly proportional to the percentage of sugar inverted.

The estimation was carried out under exactly similar conditions for every peat, the same strength of sugar solution and of Fehling's solution was used in each case.

To find the effect of treatment with N/5 HCl on the power of inverting sucrose, estimations were made both before and after acid treatment as in the case of solution by alkalies. In every case there was more inversion after acid treatment, the ratios corresponding with humus and N ratios were therefore always less than one, although in the case of acid peats they approached one very closely.

It is interesting to compare the figures for inversion after acid treatment with the total organic matter given in Table II. The highest inversion is given by the two normal peats 851 and 853 although the total organic matter is only 57·14 and 59·8 against 80·42 and 87·95 for 852 and 854. The weight of Cu_2O for 851 corresponds to an inversion of practically the whole of the sugar.

It should be noted that the abnormal peat, 850, gives the lowest inversion.

The inversion figures well indicate the acid character of 852 and 854 and the non-acid character of 851.

[1] "Über Humus säuren," *Landwirtsch. Jahrbücher*, XLI. 1911, 717.

TABLE VII.

Soil No.	Wt. of Cu_2O no acid treatment	Wt. of Cu_2O after acid treatment	Ratio $\frac{\text{no acid}}{\text{acid}}$
850	1·924	5·530	·348
851	0·439	7·111	·062
852	5·951	6·803	·875
853	1·150	6·924	·166
854	5·471	6·684	·819

The ratios between the results obtained on the various peats after and without the preliminary treatment with acid are gathered together in Table VIII.

TABLE VIII.

Soil No.	Humus ratio	N in NH_3 ratio	N in NaOH ratio	Sugar inversion ratio
850	1·222	1·203	1·087	·348
851	·112	·112	·475	·062
852	·814	·719	·964	·875
853	·647	·767	·568	·166
854	1·341	1·013	1·190	·819
	Expressed as percentages of highest ratio in each			
850	91·2	100	91·4	39·8
851	8·4	9·3	39·9	7·09
852	60·7	59·7	81·0	100
853	48·3	63·7	47·7	19·0
854	100	84·3	100	93·6

To compare the effect on plant growth of the various forms of N met with in these typical peats water cultures were used. The peats were the only source of nitrogen available for the plants growing in them. The same weight of N was added to each bottle in the form of peat itself.

Sunflowers were used and three bottles constituted a unit.

The peats 852 and 854 gave no plant at all, the roots rotted away wherever they were in contact with the solution.

851 gave a better plant than the control in which N was supplied as KNO_3.

853 was about equal to the control, and 850 not so good but grew fairly well after a doubtful start. The order of growth was the

same as the order obtained from ratios of sugar inversion, viz. 851 most fertile, then in order 853 and 850 whilst 852 and 854 refused to support plants at all and gave the highest ratios for sugar inversion.

Estimation of Acidity in Soils.

The satisfactory results obtained by the inversion of sucrose, led to a further trial of the method on other soils.

A detailed account of the method used will be given here.

In all methods for estimation of sugars, extreme care must be taken to have identical conditions as regards the manipulative part of the work.

The weight of soil taken varied from 2 gms. in the case of a peat to 10 gms. in the case of soils containing less than 10 % of organic matter.

The soil was boiled for 2 hours with 100 c.c.s of 1·25 % sucrose solution in a conical flask with a reflux condenser. A drop of neutral $CaCl_2$ solution was added to flocculate the clay and the soil was allowed to settle for an hour. It was then filtered through asbestos under pressure from a filter pump and a perfectly clear solution was always obtained even with clays.

The invert-sugar in the solution was then estimated by reduction of Fehling's solution of the strength given by Brown, Morris and Millar.

Their conditions require 50 c.c.s of Fehling's solution and 50 c.c.s of the invert-sugar solution, but in the case of acid soils 50 c.c.s of the extract is too much so a portion of 10 c.c.s or 20 c.c.s is taken and made up to 50 c.c.s with water. If possible the conditions should be arranged to give from 0·2 to 0·3 gms. of Cu_2O precipitated, though with alkaline soils the weight is usually much less.

In all cases, even with peats such as 852 and 854 of extreme acidity, the estimation was easily carried out under the conditions given above.

It was only found necessary to use such a small quantity of soil as 2 gms. in the case of peats containing high percentages of organic matter, and as these can be ground up and easily sampled the error due to sampling is eliminated to a great extent.

With soils of less than 20 % loss on ignition it was never found necessary to use less than 5 gms.

The reduction of Fehling's solution was carried out in a lipped conical beaker. The solution is first heated to boiling point in a water bath (6 minutes), then the soil extract is added and the beaker returned to the water bath for exactly 12 minutes.

It is filtered at once through a Gooch containing asbestos, which is then dried and weighed.

The weight of Cu_2O was in every case calculated as that produced by 5 gms. of soil.

In case any soil on boiling yields an extract capable of reducing Fehling's solution, duplicate samples were taken, one being boiled with water instead of sugar solution, the estimation being carried through in just the same way.

Most soils give a slight reduction in this way, and this is subtracted from the weight of Cu_2O obtained with the sugar solution. In both cases an allowance must be made for self-reduction of Fehling's solution which always takes place to a slight extent (see Brown, Morris and Millar).

Three soils losing less than 10 °/$_o$ on ignition were first tried: (1) Rothamsted soil which contains plenty of lime and may be regarded as an excellent example of a neutral or normal soil of this type with 4·2 °/$_o$ organic matter, (2) No. 422 a slightly acid soil with 8·18 °/$_o$ organic matter and (3) No. 423 a very acid soil with 7·44 °/$_o$ organic matter. All three contained a considerable proportion of clay.

In Table IX the weights of Cu_2O with and without sugar are given.

TABLE IX.

Soil No.	Cu_2O without sugar	Cu_2O with sugar	Wt. of Cu_2O precipitated by 5 gms. of soil
Rothamsted	·0050	·0093	·0043
422	·0084	·0512	·0428
423	·0106	·4593	·4487

The weight of Cu_2O (and therefore of sucrose inverted) by the Rothamsted soil is practically negligible, and the acidities are approximately in the ratio of 1 : 10 : 100.

A series of soils (containing alkaline soils) in which the $CaCO_3$ had been estimated was next selected from the list in Table I, and the results compared with the 'humus ratio' given there. The soils are placed in the order of their 'humus ratios.'

TABLE X.

Soil No.	Reaction to litmus	$CaCO_3$	Cu_2O	Humus no acid	Humus ratio	Loss on ignition
514	acid	nil	·1735	3·525	1·023	16·08
502	acid	nil	·3598	3·675	·800	14·26
450	acid	nil	·4339	4·450	·662	12·75
472	alk.	3·85	·0204	1·000	·556	11·24
470	acid	nil	·2474	1·074	·494	2·84
484	alk.	1·70	·0276	1·024	·427	8·00
500	alk.	0·30	·0291	1·075	·285	7·36

The list contains three alkaline soils, two of which contain plenty of $CaCO_3$, and although their 'humus ratios' are high, the weights of Cu_2O obtained from them are very low, ·0291 being the highest against ·1735 the lowest for acid soils.

It was found that the weight of Cu_2O precipitated directly by any soil varies greatly with the percentage of organic matter in the soil. In fact it is impossible to compare two soils with different percentages of organic matter by estimating invert sugar directly, particularly when the organic matter is upwards of 20 °/ₒ.

TABLE XI.

Normal soils			Acid soils		
Soil No.	Organic matter	Cu_2O	Soil No.	Organic matter	Cu_2O
500	7·36	·0291	423	7·44	·4487
484	8·00	·0276	427	8·27	·3869
472	11·24	·0204	450	12·75	·4339
448	40·45	·0603	850	48·75	1·924
421	74·60	·1488	852	80·42	5·951
Bl 26	27·40	·1995	514	16·08	·1735
Bl 31	57·96	·3205	502	14·26	·3598
851	57·14	·4390	424	8·77	·6554
853	59·80	1·1500	854	87·95	5·471

Table XI shows results obtained by using normal and acid soils with corresponding percentages of organic matter.

The first five in each series compare closely and in no case does the inversion by a normal soil approach that by an acid soil.

The difference made by a larger amount of organic matter is brought out by comparing the two neutral soils Bl 26 and Bl 31 whose formation and characteristics are as nearly alike as possible.

They are peats from the top of a shingle bank which is as well aerated as a soil could be. Both are formed from grassy vegetation growing on the surface of clean shingle. The only difference being that Bl 31 is higher and has been forming longer than Bl 26, as shown by its having a rather thicker turf.

Both soils contain a small amount of $CaCO_3$, higher in the case of Bl 31, and the conditions appear to be ideal for the formation of a normal (as distinct from acid) turf. Bl 26 gives ·1995 and Bl 31 ·3205 gms. of Cu_2O per 5 gms. of soil and yet, as will be shown later, their acidities are practically the same.

Just as acid soils with the same amount of organic matter vary amongst themselves, so also do alkaline soils.

The last three alkaline soils on the list contain approximately the same amount of organic matter and yet give vastly different inversions.

Again it is impossible to compare Bl 26 with 514, the former gives the larger inversion of sugar and yet is undoubtedly a normal soil whilst 514 is undoubtedly acid.

Obviously then the acidity of a soil does not depend on the actual proportion of the acid in the soil since many alkaline peats contain more acid than very acid soils of lower organic matter content.

One does not often, apart from exceptional cases of artificial manuring, meet with free mineral acid in a soil and the only other source of substances of an acid nature would be the 'humus' of the soil. This is seen when a soil is first treated with dilute HCl and then boiled with sugar solution. In the case of alkaline soils, as already shown in Table VII, the increase in inversion after a breaking down of humates is often enormous, whereas with acid soils it is very small.

It appears then as if the acidity of a soil depends on the state of the organic matter in the soil and the proportion of it which is in an acid state; but only the 'humus' will affect the acidity.

This would account for the considerable differences in inversion obtained with peats like 851 and 854 after acid treatment; although their respective percentages of organic matter are 57·14 and 87·95 the former gives the higher value for sugar inversion.

In estimating acidities then, it is necessary to know not only the proportion of humus in an acid state to the total soil, but the proportion of 'humus' as 'humic acid' to 'humus' as 'humates,' and this involves a determination of sugar inverted after treatment with dilute acid.

In addition to the five peats given above the soils in Table XII were treated in this way.

TABLE XII.

Soil No.	Loss on ignition	Cu_2O (after acid)	Cu_2O (no acid)	Ratio $\frac{\text{no acid}}{\text{acid}}$
Bl 31	57·96	5·660	0·3205	·057
Bl 26	27·40	4·693	0·1995	·043
450	12·75	2·621	0·4339	·166
472	11·24	2·108	0·0204	·0097

It appears therefore that soils with a large percentage of humus can contain a larger proportion of that humus in an acid state than soils with lower humus content, and still appear normal.

To sum up: (1) It is never safe to judge the acidity of a soil from the percentage of it directly soluble in alkalies since all soils, particularly peat, contain soluble neutral organic matter, and this will also vitiate the results obtained for the ratios of soluble to insoluble humus.

Caustic soda gives more significant results than ammonia in all cases and the results agree closely with those obtained by inversion of sucrose.

(2) The readily available proteins etc. as indicated by the yield of ammonia on hydrolysis with 20 °/。 HCl are very low in normal peats and nil in acid peats.

The method would therefore be of no use for discrimination between peats.

(3) It appears as though some less general property of acids than solubility in alkalies must be used, i.e. a reaction in which neutral compounds cannot take part, such as inversion of sucrose.

In this method the acids are neither removed nor changed directly, and the result entirely depends on the concentration of acid present.

The results obtained by inversion of sucrose agree closely with the general characteristics of the peats.

How far the results are affected by the solubility of these soil acids in the sugar solution used (the soil itself is boiled with the solution) is still a matter for investigation. But in all soils tried the alkaline or normal soils have invariably given much lower weights of Cu_2O than the acid soils and treatment with acid invariably leads to an increase in inverting power of a soil although every precaution was taken to get rid of the last trace of HCl.

Moreover normal fertile soils which one would expect to contain a large proportion of their organic matter as available humus always gave more 'total inversion' than acid soils.

THE ESTIMATION OF TANNIN IN CIDER.

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THE method at present in use for the estimation of tannin in apple juice and cider is that used by Lloyd[1]. It is a modification according to Kraus[2] of Löwenthal's[3] method, and consists in titrating the cider directly with potassium permanganate in presence of indigo as an indicator. The permanganate is standardized by means of "pure tannin," it being assumed that both this and the tannin present in the apple are digallic acid, and have the composition $C_{14}H_{10}O_9$. It is apparent that all the permanganate reducing substances present in the cider will be included in the tannin content; moreover the amount of indigo used is not sufficient to retard noticeably the oxidation of the non-tannins for which purpose it was originally used in Löwenthal's method. The results obtained by this method are therefore only comparative.

Gravimetric methods, such as the hide powder method and Wislicenus'[4] "sprouted alumina" method, which depend on the difference in weight of the residue obtained on evaporation before and after detannizing with the above named detannizing agents, are in our case of little value, since it is practically impossible to obtain a residue of constant weight by evaporation of the cider.

The methods in use by botanists are also open to criticism, and thus not suitable for the investigation of tannic acid in cider. Sanio's[5] method, which is based on the assumption that tannins are quantitatively precipitated by potassium dichromate, does not give trustworthy

[1] Lloyd, Report on researches on Cider making (Board of Agriculture), 1903.
[2] G. Kraus, *Grundlinien zu einer Physiologie des Gerbstoffs*, Leipzig, 1889.
[3] Löwenthal, *Zeit. für Anal. Chem.* 1866, 5, 838; 1877, 16, 33; 1881, 20, 91.
[4] Wislicenus, *Collegium*, 1905, 85; 1905, 213; 1905, 23; 1906, 316; 1907, 157.
[5] Sanio, *Botanische Zeitung*, 1863, 17.

results as was shown by Drabble and Nierenstein[1], since gallic acid is also precipitated by potassium dichromate. The same objection applies to a method of Kraus[2] based on potassium dichromate precipitation. Fleck[3] precipitates the tannin by means of neutral copper acetate, weighing the copper oxide resulting from the ignition of the precipitate. Sonnenschein[4] uses Fehling's solution, also weighing the copper oxide. Both these workers deduced a relation between copper oxide and "pure tannin" in order to express their results. As will be shown later, such a relation cannot be used with any certainty. The colorimetric method of Jean[5] depends upon a comparison of the tint produced by addition of ferric chloride to the solution to be examined with that produced by known concentrations of "pure tannin."

Such a comparison assumes that all plant tannins have the same constitution as gallotannic acid, which is obviously incorrect, since tannins can be divided into two groups giving green and blue colorations respectively with ferric chloride. Moreover it does not follow that solutions of different tannins which produce the same depth of coloration with ferric chloride are of the same concentration.

As was mentioned above, Kraus[6] titrated solutions directly with potassium permanganate, including all reducing substances as tannin; the content of which was calculated by standardizing the permanganate with "pure tannin." It was assumed therefore that all tannin substances are "gallotannic acid." Neubauer[7] also used potassium permanganate to estimate tannin in solutions of extracts, using, however, animal charcoal as a detannizing agent. The difference in the amount of permanganate used before and after detannizing gives a measure of the tannin present. For the standardization of the permanganate Neubauer used "pure tannin," and for convenience found a relation between "pure tannin" and oxalic acid with respect to their permanganate reducing value. The tannin content as found by this method would be somewhat too high; since other substances, notably gallic acid, are also absorbed by animal charcoal. Neubauer, however, realized

[1] Drabble and Nierenstein, *Biochem. Journ.* 1907, **2**, 96.
[2] Kraus, *Grundlinien zu einer Physiologie des Gerbstoffs*, Leipzig, 1889, p. 67.
[3] Fleck, *Deutsche Gerbzeitung*, 1860, 3, 14.
[4] Sonnenschein, *Jahresbericht für Pharmazie*, 1868, p. 150; Dingler's *Polytech. Jl.* 1885, 256, 555.
[5] Jean, *Archiv der Pharmazie*, 1885, 214, 992; *L'Union Pharmac*, 1880, 29, 548.
[6] Kraus, *loc. cit.*
[7] Neubauer, *Zeit. für Anal. Chem.* 1871, vol. 9, 1.

that this method could only give the tannin content of the solutions analysed in terms of the tannin used to standardize the solution. In his own words: "Es entsteht daher die Frage: dürfen wir bei der Gerbstoffbestimmung der Eichenrinde das Tannin zum Vergleich zu Grunde legen? Strenge genommen allerdings nicht, wenn es allein darum ankäme den absoluten Gehalt der Rinden am Eichenrindengerbstoff zu bestimmen, denn es fehlt uns ja bei der volligen Unkenntnis des letzteren in absolutem reinem Zustande all' und jede Garantie das beide Gerbsauren gleichen Mengen von Chamaleon zur Oxydation bedurfen."

Of the precipitation methods involving the use of organic bases, that of Trotman and Hackford[1] was tried. This depends upon the precipitation of the tannin by strychnin in dilute alcoholic solution. Trotman and Hackford considered the compound which they prepared from "pure tannin" to have the composition $C_{14}H_{10}O_9 . C_{21}H_{22}N_2O_2$. It will be shown that commercial "pure tannins" are mixtures which vary in composition and therefore do not give a strychnin compound of constant composition. Trotman and Hackford calculated their results on the basis of the above strychnin compound, *i.e.* they assumed tannic acid to be digallic acid. Nierenstein[2] has shown this to be incorrect, and considers tannic acid to be a complex molecule of digallic and leucodigallic acids. E. Fischer and Freudenberg[3], on the other hand, have brought forward some evidence to show that tannic acid is penta digalloyl-glucose.

It has been found that Trotman and Hackford's method does not give consistent results with gallotannic acid owing to the solubility of the strychnin compound in the dilute alcohol used, and for this reason it is not suitable at present as a standard for the case of the tannic acid in cider. The method finally adopted was a modification of that of Körner and Nierenstein[4] which consists in detannizing the solution to be analysed by means of casein, the total solids being estimated before and after detannizing. In this case, however, the tannin removed was determined by the difference in the permanganate titrations as in the method of Neubauer[5] previously mentioned, *the casein removing nothing but the tannin from the cider or tannin solution analysed*, as shown by the work of Nierenstein[6].

[1] Trotman and Hackford, *Jl. Soc. Chem. Ind.* 1905, 1097.
[2] Nierenstein, *Annalen*, 1912, 388, 223.
[3] Fischer and Freudenberg, 1912, B. 45, 915; 1913, B. 46, 1116.
[4] Körner and Nierenstein, *Chem. Zeit.* 1911, vol. 36, 31.
[5] Neubauer, *loc. cit.*
[6] Körner and Nierenstein, *loc. cit.*; Nierenstein, *Annalen*, *loc. cit.*

In the method here described a solution of potassium permanganate of about 5 grammes per litre concentration is made up and used as a stock solution. This is diluted to five times its volume as required, since such dilute solutions do not keep well. A solution of indigo carmine in dilute sulphuric acid, concentration about 5 grammes per litre, is made up: the titration is performed as in the method of Löwenthal[1]. 20 c.c. of such a solution require approximately 10 c.c. of the diluted permanganate. The titration is carried out as in the method of Löwenthal as follows: 5 c.c. of the cider or tannin solution (0·3–0·5 %) are added to 750 c.c. of water in a 1 litre shallow porcelain dish together with 20 c.c. of the indigo solution of which the permanganate titration is known.

The amount of permanganate required by the indigo compared with that used by the tannin is smaller in proportion than that suggested by some earlier workers, who used an amount of indigo which would require more permanganate than the tannin solution itself.

Neubauer observed on this point: "...bemerke ich nur dass die Indigo-lösung immer ein solche Konzentration haben muss dass 20 c.c. derselben allerwenigstens eine gleiche Menge Chamaleon wie das Tannin, oder sicherer noch die Hälfte mehr wie diese verlangen." It was found in the course of this work that quite reliable results were obtained if the fixed amount of 20 c.c. indigo solution were used. Larger amounts did not influence the volume of permanganate used by the tannin beyond the limits of experimental error. The end-point was, however, more difficult to determine with larger or smaller volumes of indigo solution.

The permanganate is run in slowly, at first about 1 c.c. at a time, with vigorous stirring for 5–10 seconds between each addition. When the blue colour of the indigo begins to disappear the permanganate is run in more and more slowly; and finally drop by drop until the liquid becomes a clear golden yellow with a tinge of pink round the edge of the dish. The appearance of this pink tinge is the most certain indication of a sharp end-point.

The titration cannot be carried out in artificial light and is best done in diffused daylight when the error is about 0·2 c.c. In actual practice at least three titrations should be made.

The detannizing of the solution is effected by shaking with casein, which to be efficient must be quite fat-free. For this work Kahlbaum's pure casein (nach Hammarsten) was used, previously extracted 36 hours

[1] Löwenthal, *loc. cit.*

with ether. It was found that for solutions of commercial "pure tannins" up to 0·5 °/₀ concentration, detannizing was completely effected by shaking 50 c.c. with two quantities of one gramme of casein for 15 minutes. The liquid is filtered before the second addition of casein; and finally before titration through a barium sulphate filter, after which 5 c.c. are titrated as before.

The permanganate used was first standardized by means of Schering's tannin leviss puriss.

It was afterwards found that different samples of the same tannin gave somewhat different figures with the permanganate, since the commercial "pure tannins" are not homogeneous substances.

A number of pure tannins were therefore titrated and the average value taken.

Tannins purified by Nierenstein's and by Fischer's methods were included for comparison.

Table I contains the tannin value of the permanganate and the tannin content of the various samples used as found by detannizing the solutions with casein. The water present in the tannin was estimated in all cases by drying to constant weight at 40° C. in a vacuum desiccator containing calcium chloride. Duplicate determinations by this method usually differed by less than 0·05 °/₀. The dried samples were afterwards used for duplicate titrations with permanganate.

For convenience in standardizing the permanganate in the future the solution used was titrated against ammonium oxalate, which is easily obtained pure and is neither hygroscopic nor efflorescent.

From this it was found, using the average value of the tannins as in Table I, that:

1 gramme ammonium oxalate = 0·4648 gramme tannin.

Table II contains the results of analyses of various ciders; mostly of the bittersweet variety. The tannin content given by direct titration with permanganate is included for comparison. These, however, are probably somewhat lower than those found by Lloyd's method, since the small amount of indigo used by him would tend to make the tannin content appear larger by the inclusion of other oxidizable substances. The titration figures given here are those of the diluted permanganate. It should be noted that since the constitution of the cider-tannin is unknown, the amount of it present in the cider is expressed in terms of the tannin used to standardize the permanganate.

Although it was found that the strychnin method of Trotman and Hackford[1] is not accurate in the case of gallotannic acid; the tannin in cider is quantitatively precipitated by strychnin after careful neutralization. This is shown by the fact that there is a parallelism between the results obtained by this method and by the permanganate titration method; although in the absence of a method of quantitative precipitation of a standard gallotannin-strychnin compound, the strychnin precipitate results cannot be expressed as gallotannin comparably with those of permanganate titration.

The preparation of the tannin of apples is now being undertaken, so that it is hoped to standardize both the permanganate solution and the strychnin precipitate by the pure apple-tannin itself.

TABLE I.

Tannin		Tannin content °/o	Tannin value of permanganate, gms. per c.c.
Schering's leviss puriss, sample	1 undried	99·36	·005450
,, ,, ,, ,,	2 ,,	95·66	·005304
,, ,, ,, ,,	2 dried	96·03	·005270
,, ,, ,, ,,	4 undried	96·61	·005516
,, ,, ,, ,,	4 dried	96·38	·005319
,, ,, ,, ,,	5 undried	94·56	·005395
,, ,, ,, ,,	5 dried	—	·005180
Merck's v. light, extra pure ,,	1 ,,	—	·005509
,, ,, ,, ,,	1 undried	94·19	·005399
,, ,, ,, ,,	2 ,,	91·24	·005388
,, ,, ,, ,,	2 dried	95·83	·005993
Kahlbaum's Gerbsaure ,,		—	—
,, ,, ,,	1 dried	93·99	·005591
,, ,, ,,	2 undried	96·05	·005526
,, ,, ,,	2 dried	96·99	·005540
,, ,, ,,	3 undried	—	·005454
,, ,, ,,	3 dried	94·64	·005623
,, ,, ,,	5 undried	96·78	·005463
,, ,, ,,	5 dried	96·61	·005921
Schuchardt's leviss puriss ,,	1 ,,	98·37	·005421
,, ,, ,,	1 undried	94·48	·005318
,, ,, ,,	2 dried	97·43	·005620
Average, used for tannin content of ciders		—	·005430
Schering's sample 3 purified by Nierenstein's method		—	·004849
,, ,, ,, ,, ,, ,,		—	·004939
,, ,, ,, ,, ,, ,,		—	·005130
,, ,, ,, ,, ,, ,,		99·77	·005216
,, ,, ,, ,, ,, ,,		99·70	·005117
,, ,, ,, ,, ,, ,,		99·42	·005170
,, ,, 1 ,, ,, ,,			
Schering's sample 5 purified by the method of E. Fischer and Freudenberg		—	·005554

[1] Trotman and Hackford, *loc. cit.*

TABLE II.

Cider	S.G.	Total $KMnO_4$	Tannin $KMnO_4$	Tannin by direct titration	Tannin by casein method
Newton St Cyres, bottled 7.2.12	1·035	18·26	14·65	0·38	0·31
Early Red Jersey (1), ,, 1.12.11	1·018	26·17	21·40	0·56	0·46
,, ,, (2), bottled 1.12.11	1·007	27·35	21·83	0·59	0·47
Research Station Dabinett, bottled 15.1.12	1·028	11·48	9·09	0·24	0·19
Frederick B, bottled 7.2.12	1·097	7·06	7·68	0·14	0·10
Twistbody Jersey (1), bottled 16.1.12......	1·037	31·95	29·10	0·67	0·50
,, ,, (2) ,, ,, ...	1·033	30·87	24·58	0·65	0·52
Sam's Crab, bottled 20.1.12..................	1·031	7·70	5·80	0·16	0·12
Wallis' Red, ,, 6.2.12	1·025	8·85	8·55	0·19	0·18
Farmer's Friend, bottled 5.1.12	1·029	4·21	2·61	0·09	0·05
Ashton mixed, bottled 1912..................	1·001	17·45	13·99	0·37	0·28
Glastonbury Jersey, bottled 15.2.12	1·030	27·84	22·09	0·59	0·46
Neverblight, bottled 15.2.12..................	1·018	12·37	9·66	0·26	0·20
Farmer's Friend, bottled 5.1.12	1·019	2·87	1·76	0·06	0·04
Ashton White, bottled 1912..................	1·001	12·61	10·02	0·27	0·21
Neverblight, bottled 15.2.12..................	1·018	12·12	9·44	0·26	0·20
Sweet Alford, sick, filtered 1.	1·035	8·57	6·04	0·18	0·12
Newton St Cyres, sick, filtered	1·023	12·85	10·50	0·26	0·22

THE SOLUTION AND PRECIPITATION OF IRON IN THE FORMATION OF IRON PAN.

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THE formation of Iron Pan or Ortstein is of fairly frequent occurrence both in Britain and on the Continent of Europe. It is of some economic importance, for where it occurs considerable expense has to be incurred before it is possible to grow a crop of any kind on the soil. The Pan consists of a hard layer of material which has to be broken through for successful cultivation, as it is cemented together in such a manner as to prevent any penetration of plant roots and to limit the circulation of air and water.

It does not appear to be associated with any one physical soil type, although it is certainly commoner on soils containing much sand and coarse material, but formations of a similar character are to be found on soils of a heavier nature.

Associated with Pan formation are certain distinctive soil conditions. In the first place there must be an almost complete absence of calcium carbonate. This results in a considerable tendency for the accumulation of acid humus on the surface, the formation of which will be encouraged or hindered by position and facilities for drainage.

This humus layer varies from 5–20 cms. in thickness, and the most conspicuous plants associated with it are whortleberry and various heaths. Immediately beneath this humus covering lies a layer of sand from which most of the colouring matter has been removed, and which has been bleached in exactly the same way as a stone embedded in a peat bog has been bleached, owing to the removal of ferric hydroxide to which yellow sand normally owes its colour.

The other most important characteristic of this layer is its poverty in soluble mineral material. Many years' exposure to the liquid

draining through the layers of peat on the surface have removed these bodies. This layer of bleached sand may vary in thickness from 5–60 cms.

At a certain depth, which probably varies with the level of the soil water in summer, there is a sudden change to the Pan proper. This may vary in thickness from 10 to 60 cms., the variation being probably caused by the amount of soluble material in the soil above.

The Pan consists of a hard mass quite impervious to water and to the roots of plants, and which varies in colour from yellowish-brown to black, according to the amount of organic matter which it contains. As the amount of organic matter decreases, the colour changes until the yellow brown of the ferric hydroxide becomes the dominant shade.

Analyses of various Pans are given in Table I.

These figures show conclusively that there is a great concentration of organic matter in the Pan and that of the soluble minerals the most important are iron, aluminium, and silicon.

There has been a great deal of work done on the subject, and various theories have been put forward to account for the formation of the Pan. A list of the more important contributions to the subject is given at the end of the present paper.

In the main the result of these papers is to put forward three distinct theories for the formation of Iron Pan, with which the names of Mayer, Faye, Münst, and Ramann, may be respectively connected.

The first of these theories is that which traces Pan formation to the alternate reduction and oxidation of iron humates in which the iron on the surface is reduced by the acid humus to the ferrous state dissolved as ferrous humate carried down to the subsoil, and there precipitated as ferric humate owing to oxidation by the air which at the dry season can readily penetrate to that depth.

The second explanation is that which is given in *The Soil*, A. D. Hall, in which it is supposed that the organic matter of the soil will reduce ferric oxide to ferrous oxide, and then that the carbon dioxide solution in the soil will cause its solution as ferrous bicarbonate, which is reconverted into ferrous carbonate at the Pan level, and probably later on oxidised to the ferric state.

Lastly the more modern papers on the subject consider the formation of Pan to be due to the formation of colloidal humus compounds of iron and aluminium, which are carried down into the soil, and are there precipitated by soluble salts, by loss of water, or by the change of bases.

It will be obvious in the course of this paper that the authors are

in agreement with the last of these explanations, and it was largely because the experimental work on which these conclusions were based seemed in many cases unsatisfactory that they decided to re-examine the whole subject.

Table I.

1. *Caesar's Camp.*

		Bleached sand	Pan	Soil below Pan	
	Hyg. moisture	1·27	3·064	·902	% in soil
	Loss on ignition	1·84	7·220	1·36	
Soluble in hydrochloric acid	Fe_2O_3 and Al_2O_3	·493	4·066	3·211	
	CaO	·0801	·350	·106	
	MgO	·0632	·0841	·1105	
	P_2O_5	trace	·0366	·0183	
	K_2O	·0870	·1546	·1520	

2. *Freudenstadt, Schwarzwald.*

		Bleached sand	Pan	Soil below Pan	
	Hyg. moisture	·300	3·79	·768	% in soil
	Loss on treatment NH_4NO_3	1·577	10·92	1·946	
Soluble in hydrochloric acid	SiO_2	·312	2·698	1·527	
	Fe_2O_3	·253	1·857	·906	
	Al_2O_3	·180	4·946	1·268	
	P_2O_5	·029	·059	·043	
	CaO	trace	·019	·021	
	MgO	·015	·118	·088	
	K_2O	·035	·172	·052	
	Na_2O	·011	·041	·016	

3. *Schwarzwald.*

		Bleached sand	Pan	Soil below Pan	
	Hyg. moisture	·084	1·156	·341	% in soil
	Loss NH_4NO_3	·363	3·566	·813	
Soluble in hydrochloric acid	SiO_2	·347	1·253	·647	
	Fe_2O_3	·038	1·811	·592	
	Al_2O_3	·132	1·426	·695	
	P_2O_5	·023	·154	·092	
	CaO	·026	·029	·025	
	MgO	·009	·049	·022	
	K_2O	·035	·184	·108	
	Na_2O	·009	·019	·016	

4. *Schwarzwald.*

		Bleached sand	Pan	Soil below Pan	
	Hyg. moisture	·341	2·450	·400	% in soil
	Loss NH_4NO_3	1·072	7·032	·845	
Soluble in hydrochloric acid	SiO_2	·572	1·123	1·155	
	Fe_2O_3	·193	·767	·552	
	Al_2O_3	·455	2·133	·975	
	P_2O_5	·069	·091	·128	
	CaO	·031	·036	·029	
	MgO	·011	·025	·031	
	K_2O	·083	·120	?	
	Na_2O	·022	·040	·030	

Analyses 2, 3 and 4, Münst, Abstract in *Zentralblatt für Agrikulturchemie*, Jan. 1912.

It having been the custom to regard iron as being the effective agent in forming the Pan, and as most of the explanations of this action revolve round alternate reduction and oxidation of iron compounds, it

was decided to investigate these bodies. The delicacy of the colour reactions of the ferrous and ferric ion offers facilities which are not forthcoming in the case of aluminium. It was only at the close of this investigation that it became apparent that aluminium plays a no less important part than iron in the formation of the Pan.

The experimental work has been mainly concerned with the investigation of the properties of various so-called humates and with attempts to dissolve iron either as ferrous humate or as ferrous bicarbonate under conditions which approximate to those which naturally occur.

It was also hoped to obtain some information as to the way in which iron so dissolved was precipitated to form the Pan.

Various humates were prepared in the way recommended by Mayer, which consists in adding to humic acid, dissolved in the least possible quantity of alkali, solutions of salts of the desired metals. In this way ferrous, ferric, and calcium humate were prepared, and the precipitates obtained were subjected to prolonged washing. In each case the precipitates were not entirely insoluble, but gave solutions containing both the base and organic matter.

The terms humates and humic acid are used by the authors purely for the sake of convenience, and they do not wish to express an opinion as to their existence or otherwise.

It is entirely unimportant, from the point of view of the present work, whether so-called acid humus contains a definite acid, or whether it is a colloidal absorption complex showing some of the reactions associated with acids.

After 5 days' washing, the filtrate from the ferrous humate still gave deep blue colorations with ferricyanide. After the same period, the washings from the ferric humate also gave a deep coloration, although all traces of chloride from the ferric salt employed had long disappeared. It therefore appears that ferric humate cannot be prepared pure by the above method, and that ferrous humate is to a certain extent soluble, and that the solution is capable of giving ferrous reactions.

The question of the method of solution and consequent precipitation of the iron was next investigated.

It is stated by Mayer that perfectly healthy soils, provided that organic matter be present, will give the reaction of ferrous iron with potassium ferricyanide when boiled with dilute hydrochloric acid. This was tested by boiling a little healthy soil with hydrochloric acid in the presence of peat. A strong reaction of ferrous salts was obtained.

As solutions containing ferrous iron may be extracted from peat itself by the action of hydrochloric acid, the experiment was repeated, the peat being replaced by peat which had already been boiled with hydrochloric acid and then well washed. Again the reaction for ferrous salts was obtained. A little of this purified peat was then shaken with a solution of ferric chloride. Reduction commenced immediately, even in the cold. The first experiment was then repeated, the hydrochloric acid being replaced by sulphuric acid, citric acid, ammonium chloride, ammonium oxalate, ammonium carbonate, potassium chloride, and sodium chloride. In the first three cases the ferrous reaction was easily obtained. With the ammonium salts of weak acids, brown solutions incapable of being tested were obtained. With potassium chloride there was a very slight reaction: with sodium chloride, and with a simple mixture of peat and soil, no reaction was obtained.

TABLE II.

		°/₀ ammoniacal nitrogen in air-dry peat
I.	Peat	(i) ·004395
		(ii) ·004547
II.	Peat, deep Irish bog	(i) ·003860
		(ii) ·004307
III.	Peat, medium depth Irish bog	(i) ·01162
		(ii) ·01123
IV.	Acid humus from surface of Pan	(i) ·0004297
		(ii) ·0004676
V.	Acid humus from surface of Pan	(i) ·001108
		(ii) ·001106
		(iii) ·001108

The experiments were then repeated with the substitution of precipitated ferric hydroxide for the soil. The results obtained in each case were exactly similar.

A still further set of experiments was made using as a source of iron the mineral limonite very finely ground. In this case the effect of the presence of carbon dioxide and peat was investigated both under ordinary conditions and under sterile conditions obtained by the addition of 1 c.c. of chloroform to each bottle.

The details of the experiment are not given, as there was no solution of ferrous compounds in any case.

These experiments seemed to prove that though the peat had a strong reducing action on any iron salts present, it had no direct action on ferric oxide itself, unless some body capable of bringing small quantities of iron into solution were present.

The experiments further show that the usual methods of determining whether ferrous salts are present in a soil—i.e. by extracting with some strong acid—are quite useless if organic matter is present. Further work is in progress on this subject.

As ammonium chloride was by far the most efficient salt in bringing about the reaction described above, it seemed possible to the authors that ammonium salts present in the peat might play some part in aiding the solution of ferric oxide of the soil previous to its reduction to ferrous compounds.

The following determinations of ammoniacal nitrogen in peat and acid humus were therefore made, after the method described by Russell[1].

The ammoniacal nitrogen is in most cases very many times greater than that found in ordinary soils.

Litre bottles were then taken and filled with the following solutions:

(1) Precipitated ferric hydroxide and 750 c.c. distilled water, saturated with carbon dioxide.

(2) Precipitated ferric hydroxide and 750 c.c. distilled water, and the humic acid prepared from 15 gms. of peat.

(3) Precipitated ferric hydroxide and 750 c.c. water, saturated with carbon dioxide and humic acid as in (2).

(4) Entirely similar to (3).

(5) 100 gms. soil containing iron, 20 gms. peat washed free from ferrous salts, 1 gm. ammonium chloride and 750 c.c. carbon dioxide water.

(6) Similar to (5) with the omission of ammonium chloride.

The bottles, with the exception of number 4, were placed in a mechanical shaker. No. 4 was placed in a thermostat at 18° C.

The bottles were removed from time to time. 25 c.c. were removed, filtered, and examined for the presence of ferrous iron.

No trace of ferrous iron was found after 1, 2, and 3 weeks.

Bottles 3 and 4 were then also placed in a thermostat at 20° C. with an addition of 1 gramme of ammonium chloride to 4, and all were examined after 5 days with the same negative result.

In this way it was shown that no iron is dissolved in the form of the ferrous ion, and that if it is removed in the form of a ferrous humus compound that compound is unlike the one prepared in the experiment already described which gave continually the reaction of ferrous iron.

[1] *Journal of Agricultural Science*, Vol. III.

The solution from bottle 5 was examined by boiling with dilute potassium hydroxide to determine whether all the added ammonium chloride had been absorbed by the soil, and it was found that there was a considerable quantity in solution. At the same time a brown precipitate was obtained. This precipitate was dissolved in boiling dilute hydrochloric acid, and tested with a solution of ammonium sulphocyanide for the presence of ferric iron.

A faint red colour was obtained. If the precipitate was dried and ignited and then dissolved in dilute hydrochloric acid, and if it were allowed to stand for some time in contact with the concentrated acid, the reaction was much more intense.

A similar precipitate but smaller in quantity was obtained from bottle 6.

The solution from bottles 2, 3, and 4 gave no precipitate with potassium hydroxide, but the solution on evaporation and ignition, and subsequent solution in hydrochloric acid, gave the characteristic red colour with ammonium sulphocyanide.

On evaporating liquid from the bottles 1–6, that from 6 was the only one to give a precipitate with increasing concentration, which was shown to consist of ferric hydroxide. If the precipitate be filtered off and the filtrate again evaporated, a residue is obtained similar in kind to that obtained from the other bottles. It consisted of a brown sticky substance sometimes resoluble in water and sometimes not, with no apparent regularity.

On ignition of this residue ferric iron was found to be present in all cases, but most abundantly in 6, 5, and 2.

It appears from these results

(1) That ammonium chloride has no effect in bringing into solution any iron in the form of either the ferrous or ferric ion; but that in bottles 5 and 6 of the last experiment the amount of iron in solution has been in some way affected.

(2) The iron, though apparently in solution, gives none of the reactions of the ferrous or ferric ion, consequently it is either part of a complex ion or it is present in an iron humus compound in the form of a colloid sol.

The following experiments were made in the hope that some comparison of the actual amount of iron removed might be made.

(1) Washed peat, 75 gms. soil, 750 c.c. of distilled water saturated with carbon dioxide.

(2) Similar to (1).

(3) Washed peat, 75 gms. soil, 750 c.c. carbon dioxide water, 0·5 gm. ammonium chloride.

(4, 5, 6) Similar to (1), (2) and (3) with the soil in each case replaced by precipitated ferric hydroxide.

In these cases the soil used was the subsoil from the Freudenstadt Pan.

Bottles 2 and 4 were placed in a thermostat at 35° C., the others were left at the room temperature. At definite intervals 25 c.c. solution were filtered off, evaporated, ignited, and dissolved in hydrochloric acid. The solution was then compared with a solution containing a known quantity of ferric iron, 5 c.c. of an ammonium sulphocyanide solution being added to each. Equal volumes were taken in each case and placed in similar glass cylinders. The standard solution was then added from a burette until approximately the same tint was reached. The standard solution contained ·00085 gm. ferric iron per c.c. and the results below are expressed in c.c. of standard solution.

	After 7 days	14 days	21 days
1.	0·33	0·30	0·30
2.	0·15	0·16	0·20
3.	0·30	0·25	0·30
4.	0·12	0·20	0·18
5.	0·10	0·12	0·10
6.	1·04	1·40	1·50

The results are left expressed as c.c. of standard solution, as, although attempts were made, this colour method seemed too inaccurate for the determination of small amounts of iron, for the results, obtained by using different standard solutions, showed very little agreement.

Although it is impossible to place reliance on the above figures as representing the amounts of iron removed, there are some interesting points to be noted in connection with them. Both in this and in the earlier experiments the amount of iron removed in the case of the mixtures kept at higher temperatures is the lowest, which is what would be expected if the solution is in reality a colloidal suspension.

The amount of iron in five cases out of the six is of the same order, while in the last bottle it is many times greater. Thus ammonium chloride, while it has no effect on the amount of iron removed from the soil, considerably increases the amount dissolved if ferric hydroxide be substituted for the soil.

As the soil in the above experiments was the normal sand from Freudenstadt it is quite reasonable that this which had been for some

time air dry should be more resistant than freshly precipitated ferric hydroxide.

It is possible to explain the increase caused by ammonium chloride as being due to the formation of ferric hydroxide sol. It is well known that the sol only exists in the presence of small quantities of ferric chloride, which may be formed owing to the hydrolysis of the ammonium chloride. The observed increase may be either due to the ferric hydroxide sol itself or to an increase in case of formation of ferric humate sol.

It will be observed that the results do not agree with the results which were obtained previously. In bottles 5 and 6 of the earlier series of experiments it was found that an addition of ammonium chloride caused a diminution in the total amount of the iron removed. The explanation of this fact probably is connected with the nature of the soil which was employed. The authors at that period of their investigation, having no sample of sand from a Pan formation, used as a source of iron a red Devonshire soil which happened to be in their possession and which seemed to be most suitable. It represented a soil in a high state of fertility, and consequently on shaking with a saturated solution of carbon dioxide and ammonium chloride, a considerable amount of various materials would be dissolved. This solution would be a comparatively concentrated one, and the formation of the various sols would proceed with some difficulty.

The authors therefore regard the great difference between the amount of soluble material in the Freudenstadt sand and the Devon soil as explaining the result which was obtained with the latter.

According to Tacke and Suchting[1], metallic iron dissolves in the presence of peat and water with evolution of hydrogen. It seemed of interest to repeat this experiment and further to investigate the state of combination of the iron that had been attacked. A mixture of peat, distilled water, and pure iron filings, was heated on the water bath. Hydrogen was evolved in fairly large quantities. After some time the liquid from the mixture was filtered off. A solution was obtained giving a strong ferrous reaction with potassium ferricyanide, and resembling in every way the solution of ferrous humate described above.

A portion of this solution was then oxidized by being gently warmed with hydrogen peroxide. A slight brownish precipitate was obtained, and was filtered off. The solution was tested both with

[1] *Landwirtsch. Jahrbuch.* 1911, p. 717.

potassium ferricyanide and ammonium sulphocyanide, but neither ferrous nor ferric iron could be detected. If, however, a portion was evaporated to dryness, ignited, and re-dissolved in hydrochloric acid, the ferric reaction was at once given. The precipitate, either on standing with concentrated hydrochloric acid, or on ignition and solution in dilute acid, also gave a distinct ferric reaction.

It appears, therefore, from the similar behaviour of the solution obtained from the bottles and that obtained by the oxidation of ferrous humate, that the same compound is present in each case. Whether this solution is a colloidal solution of true ferric humate, or a colloidal absorption complex of colloidal humus and colloidal ferric hydrate, such as Baumann[1] and Gully consider to be formed in all so-called humates, is not quite clear.

Finally, 250 c.c. of the solution in bottle 3 of the last series containing peat, soil, and ammonium chloride, were evaporated to dryness on the water bath. The residue obtained was only very slightly soluble in water. The residue was strongly ignited and weighed. It was then dissolved in hydrochloric acid and analysed. It consisted mainly of iron, aluminium and calcium, with slight traces of magnesium.

Total weight of ash......	·0260 gm.
Fe_2O_3 and Al_2O_3	·0092 ,,
CaO	·0144 ,,

From these experiments the following conclusions may be drawn:

I. That peat is a strong reducing agent, but is not capable of reducing ferric oxide to ferrous oxide. The facts therefore seem in disagreement with the theory of Faye.

II. That the solution obtained by the action of peat on ferric oxide does not contain ferrous humate, which appears to be accompanied by the presence of ferrous ions. It therefore seems that Mayer's theory of the formation of Iron Pan is not correct.

III. That peat in the presence of water removes considerable quantities of minerals, especially ferric oxide, aluminium oxide, and calcium oxide, from the soil as colloidal suspensions. These colloid sols do not seem very sensitive to changes in concentration. Evaporation to dryness, however, destroys, at any rate to a certain extent, their capacity for suspension.

[1] "Mitt. d. K. Bayr. Moorkulturanst." 3. (52–123), abstract in *Jahresbericht über... Agrikultur Chemie*, 1909, XII. p. 52.

IV. That in the case of iron, the compound formed is probably ferric humate, but possibly an absorption complex of colloidal humus and colloidal ferric hydroxide.

As a result of the above experiments the authors believe that none of the previous theories completely represents the conditions obtaining during the removal of iron, and they put forward the following as being the most probable course of events.

One of the first results of the accumulation of the surface layer of peat is the production of substances showing acid properties.

The first action of these will be to remove the constituents of the soil which are more readily attacked. These are probably in the state of true solution. At the same time there are formed colloidal humates of iron, aluminium and calcium.

Owing to the fact that at the commencement the solution in the soil is comparatively of high concentration, these colloids are probably in the gel form. They do not assume the sol form until the concentration of the solution is much lower.

This would explain the fact that Pan formation is more common on sandy soils than on clays. The latter containing much more soluble material, owing to their composition and their large surface development, hinder the formation of the sol condition of these colloids and consequently the materials which form the Pan are not removed from their old position.

As soon then as the formation of these sol forms is possible, the iron and the aluminium and the calcium will be removed from the layer of what ultimately becomes bleached sand. Probably they are removed in the order calcium, aluminium, iron, as from the authors' experiments the concentration of ferric humate sol is smaller than calcium humate sol. This ferric humate may be formed by direct union of humic acid and ferric hydrate, or by the precipitation by ferric hydrate of solutions of other humates, such as the calcium salt. In the presence of ammonium chloride small quantities of ferric hydrate sol might be formed which would probably assist both reactions.

In the preparation of humates described above, the fact that as the wash water removed more and more salts so the humate gel tended to pass into the humate sol, lends support to the above.

It is just worth remarking here that the sol form of this particular colloid seems to be remarkably stable. Coagulation with electrolytes only occurs with great difficulty, and boiling does not

always appear to bring about this result. Further investigations are also contemplated on this subject.

The authors then believe that as the concentration diminishes, ferric humate and the other colloidal humates tend to pass into the sol form. This is what is occurring throughout the wetter season of the year.

As the soil dries up and the water recedes from the surface, the major part of the colloidal suspension is taken with it, the water becomes more concentrated with respect to the colloid, and a small amount of the colloidal humate will be left behind in what will ultimately become the bleached sand layer.

When the desiccation process has progressed still further to a point about the level of the permanent water table in the soil, then the amount of ferric humate deposited will be larger.

The great bulk of the sol will have accumulated at this level and, owing to the negligible osmotic pressure of the colloid sol, it is certain that very little diffusion will take place throughout the mass of soil water with which it is now in contact. Consequently the process of desiccation will be much more rapid than diffusion, bringing about a deposit of practically the whole of the material which was in suspension.

The condition of affairs at the end of the wet season is then briefly this, that throughout the layer to be converted into bleached sand there is a small amount of ferric humate and a considerable deposit of it at a lower level just above the permanent water table.

As soon as the deposit at this level exists at all, it is easy to imagine the rapid manner in which it will increase, and it is not difficult to conceive of the actions described going on from year to year until the result that is called Iron Pan is reached.

On the arrival of the wet season again the coagulated and desiccated colloids will not entirely go back into suspension, as the colloid character may well have been changed during the process of desiccation.

It is also probable that oxidation plays a part in establishing the stability of the Pan. The air in summer will have comparatively free access down to the level at which the Pan is formed. Oxidation cannot affect ferric iron, but it will affect the humic acid, which will be partially oxidized; some of it will be converted into carbon dioxide and water, some will be left behind as a residue which is richer in oxygen than the original humic acid[1].

As time goes on the oxidation will proceed further and further till most of the iron will be left in the form of ferric hydroxide.

[1] Mayer, *Landwirtschaft. Versuchstat.*

The original humic acid of the bleached sand represents, as it were, one year's deposit, and is not subjected to prolonged oxidation in that position, being gradually transferred to the Pan layer.

In the presence of ammonium chloride it is also possible to get small quantities of ferric hydrate sol formed. This may play some part in the removal of iron. The same cycle of reactions would occur with this as with any other colloid sol. This is the only effect due to ammonium salts at ordinary temperatures of which any evidence was obtained.

It is conceivable that some of the iron bacteria may play a part in the formation of Pan. During the summer they will be most active at the level of the Pan, because that will be the only layer which will hold enough moisture for their existence.

It is possible that, in the absence of much organic matter, organisms might use the humic acid combined with the iron as a source of energy, and leave the iron in the form of ferric hydroxide. The authors, however, consider that it is possible to account for the formation of Pan without the intervention of living organisms. They are in complete agreement with the conclusions of Münst with regard to the *rôle* of colloidal humates, and would only emphasize the probable importance of iron as well as aluminium.

In conclusion, the authors wish to thank Sir William Schlich, F.R.S., for having very kindly obtained for them eight samples of material, four from Freudenstadt and four from Cooper's Hill.

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FERROUS IRON IN SOILS.

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In a previous paper to this Journal by one of us it was observed that if a solution of a ferric salt be placed in contact with peat from which all the ferrous salts had been previously removed by boiling with hydrochloric acid, there was an immediate production of ferrous iron in the solution.

This fact and the statement by A. Mayer[1], that perfectly healthy soils on treatment with a dilute acid gave abundantly the reaction of ferrous ion, indicated that the ordinary methods for determining ferrous iron in the soil were not reliable.

These methods[2] consist essentially in the use of a dilute acid as a solvent and the subsequent titration of the solution so obtained with potassium permanganate, observing certain precautions on account of the colloidal organic matter which goes into suspension.

The ferrous iron as indicated by the above method was determined in the four following soils:

(1) A clay pasture soil containing 8·9 °/$_0$ of calcium carbonate.

(2) A clay pasture soil containing 0·6 °/$_0$ calcium carbonate.

(3) A sour pasture soil.

(4) A sandy arable soil in good condition containing practically no calcium carbonate and giving a slightly acid reaction.

10 grams of each were digested in a water bath for two hours with 100 c.c. of dilute sulphuric acid (1 part of acid to 3 of water); this was then made up to 250 c.c. filtered and an aliquot part titrated with potassium permanganate. The results are given in Table I.

[1] *Landwirt. Versuchstat.* LVIII. p. 161.

[2] *König. Untersuchunglandwirtschaftlich und Gewerblichwichtigestoffe*, p. 41.

Table I.

Soil 1	1·09 %	ferrous iron in air-dry soil
,, 2	1·72	,, ,, ,,
,, 3	1·29	,, ,, ,,
,, 4	0·26	,, ,, ,,

These figures show quantities of ferrous iron amounting in the higher cases to something like 30,000 lbs. per acre.

If ferrous iron is as toxic to plants as it is commonly supposed to be this large quantity must be present in the soil in a highly insoluble form.

Of the four soils experimented with, no. 3 is the only one which is infertile, nos. 1 and 2 carrying a very good herbage.

The authors therefore believe that these large amounts of ferrous iron are indicated, not because of any large amount really present in the soil, but because the ferric iron which is dissolved by the acid is partially reduced by the organic matter present.

That acid peat can very readily cause reduction may be demonstrated as already mentioned, by warming a ferric chloride solution with peat, which has been well washed with hydrochloric acid to remove ferrous salts; on filtering the solution the characteristic reaction is obtained with a solution of potassium ferricyanide.

To confirm this result the following further experiments were made. Soil no. 4 was treated as in the first experiment with the addition of 0·25 gram of washed peat in the one case and 0·5 gram in the other.

The results are shown in Table II.

Table II.

Soil 4	0·26 %	of ferrous iron in air-dry soil
+ 0·25 gm. of peat	0·83	,, ,, ,,
+ 0·5 ,,	1·35	,, ,, ,,

This experiment was repeated using modelling clay and adding to it varying quantities of peat.

The results are given in Table III.

Table III.

Expt. 1	10 gm. clay 200 c.c. 2N Sulphuric acid			0·062 % Fe″
,, 2	,, ,, ,,	+ 0·25 gm. peat	= (2·5 % org. matter)	0·089 ,,
,, 3	,, ,, ,,	+ 0·5 ,, ,,	= (5·0 ,, ,,)	0·12 ,,
,, 4	,, ,, ,,	+ 0·75 ,, ,,	= (7·5 ,, ,,)	0·19 ,,
,, 5	,, ,, ,,	+ 1·0 ,, ,,	= (10 ,, ,,)	0·29 ,,
,, 6	,, ,, ,,	+ 2·0 gms. ,,	= (20 ,, ,,)	0·39 ,,

When portions of the solutions were treated respectively with

ammonium sulphocyanide and potassium ferricyanide, the intensity of the colour with the former decreased from 1—6 and with the latter increased from 1—6.

These experiments show clearly the manner in which the amount of ferrous iron determined depends on the quantity of organic matter which the soil contains. It is also probable that the character of the organic matter affects the reaction, but owing to the present lack of information on the subject it is impossible to say in what way.

It is evident therefore that the method breaks down because the amount found depends on three independent factors:

(1) the amount of ferrous iron present,

(2) the amount of soluble ferric iron present,

(3) the amount of organic matter present.

These methods being of no service the authors thought that it might be possible to use acetic acid for the purpose of dissolving out the ferrous iron and leaving at the same time the ferric iron unattacked. Qualitative experiments showed very small amounts of ferrous iron dissolved in the case of soils 1 and 2, a blue subsoil clay on the other hand showed ferrous iron in plenty, as did also the modelling clay used in the other experiments with hydrochloric acid.

The method was essentially the same substituting acetic acid for hydrochloric and titrating with one hundredth normal potassium permanganate.

The results are unsatisfactory owing to the small amounts of ferrous iron dissolved and to the large quantity of colloidal organic matter in suspension and the fact that a solution of potassium permanganate of this strength is not a satisfactory indicator. The figures are given in Table IV.

TABLE IV.

Soil 1	0·043 % Fe''	Soil 3	0·086 % Fe''
,, 2	0·086 ,,	,, 4	0·004 ,,

For this method to be satisfactory it must be shown that it removes all the ferrous iron present in the soil, and leaves the ferric iron unattacked. In order to settle the first point 100 grams of soil 3 were treated with double normal acetic acid instead of normal, and at the end of the first extraction the soil was filtered and the extraction was repeated.

The soil was extracted four times in all; 10 grams of washed peat were added the fourth time. The results are given below.

TABLE V.

Soil 3.	1st extraction.	0·125 % of ferrous iron in air-dry soil	
	2nd ,,	0·057 ,, ,, ,,	
	3rd ,,	0·037 ,, ,, ,,	
	4th ,,	0·133 ,, ,, ,,	after 10 gms. of washed peat had been added

The alteration in the mass of soil and in the concentration of the solvent has shown a different percentage of ferrous iron in the soil, and two further extractions have removed further quantities. These two facts show that the whole of the ferrous iron is not removed by the acetic acid, and the result of the fourth extraction makes it probable that the ferrous iron determined in the first three extractions was ferric iron subsequently reduced.

This probability is strengthened by the fact that it is possible to obtain a slight ferric reaction on treating soil with dilute acetic acid, and by the following experiment with soil 1.

TABLE VI.

Soil 1.	Treated with N acetic acid	.. ..	0·004 % Fe″
	,, ,, ,,	and 0·25 gm. washed peat...	0·016 ,,
	,, ,, ,,	0·50 ,, ,, ...	0·019 ,,

This method had also to be abandoned, it having merely served to indicate that the amount of soluble ferrous iron must in the case of most healthy soils be very small.

As there is very little knowledge of the way in which soils behave towards soluble ferrous compounds the authors made the following experiment: 10 grams of the four soils were shaken for 16 hours with 500 c.c. of a solution of ferrous ammonium sulphate, and the amount of ferrous iron determined before and after treatment with the soil.

TABLE VII.

500 c.c. of a solution of ferrous ammonium sulphate contained	0·0745 gm. Fe″
500 c.c. of this solution after shaking with 10 gms. of soil no. 1 contained	0·0015 ,,
,, ,, ,, ,, ,, 2 ,,	0·0279 ,,
,, ,, ,, ,, ,, 3 ,,	0·0651 ,,
,, ,, ,, ,, ,, 4 ,,	0·0223 ,,

No conversion into ferric iron had occurred in any case and it is interesting to note that an equivalent amount of calcium had gone into solution.

It would appear from these experiments that ferrous iron has been retained by the soil in the same manner as potassium. It is difficult to explain the behaviour of soil 3. It is a clay containing a high percentage of organic matter, is sour, and in spite of the large amount of

colloid material it contains it shows very little power of absorbing ferrous iron.

This may possibly be due to some alteration in the clay colloids caused by the acid conditions or to the fact that in this case they are already really saturated with the ferrous ion.

The fact that if a solution of ferric chloride be treated with some washed peat immediate reduction occurs, led the authors to experiment with various soils and a dilute solution of ferric chloride.

The concentration of the solution of ferric chloride was one two-hundredth normal and, of the eight soils used, two were carboniferous limestone soils, one of which contains a large amount of neutral humus, a sour peat, and a clay soil containing about 5 °/。 of calcium carbonate. The other four were the soils used in the former experiments.

200 c.c. of the ferric chloride solution were shaken with 100 grams of soil in every case except the acid peat, where 50 grams were taken.

After the solutions had been shaken, they were filtered and it was found that in no case was there any iron remaining in solution either in the ferrous or ferric condition.

A small amount of iron was detected in suspension in the form of a colloid sol. From this dilute solution of ferric chloride the iron was probably precipitated in the form of ferric iron humus compound.

When a soil is shaken with a strong solution of ferric chloride, part is absorbed, part is reduced to the ferrous state and part is unaltered.

In the face of these experiments it is rather difficult to understand in what form iron reaches the plant. Further work is contemplated on this part of the subject. It is also proposed to investigate fully the relation of soils to solutions of both ferrous and ferric salts.

The results of this present work may be summarised as follows:

(1) No known method is satisfactory for the determination of ferrous iron in soils.

(2) The existence of ferrous iron in normal soils to any extent seems improbable.

(3) The existence of the ferric iron in the normal soil solution seems just as unlikely.

PRELIMINARY INVESTIGATION INTO THE VARIATION IN THE PHYSICAL COMPOSITION OF WHEAT MILLING OFFALS.

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THE various offals obtained from the milling of wheat for flour have long been used as a feeding stuff for cattle, but their relative value as a food has never been thoroughly investigated.

The chief reason for this lack of investigation has been the varying nature of these offals. This variation in composition not only occurs in the products obtained from different mills widely situated from one another, but one particular mill does not necessarily always turn out offals of even approximately standard qualities.

This investigation has been taken up with the approval and help of the Agricultural Education Association, the main objective being to find out within what limits wheat offals on the market vary as regards their physical composition. Further, it was hoped to devise rapid and simple methods for measuring this variation, and at the same time to ascertain if it would be possible to formulate standards for wheat offals.

With the kind help and cooperation of many agriculturists and millers I collected about 100 authenticated samples and subjected these to examination.

My first point was to differentiate between the samples and separate them into classes according to appearance, this obviously being the simplest method of grading. Subsequently I endeavoured to evolve tests which would follow as far as possible this initial classification.

Appearance.

By comparing the offals one with another I was able to arrange them in a series as regards fineness and whiteness. There were no strong points of demarcation however visible in this arrangement, the samples following one after the other with hardly any appreciable difference between succeeding samples. In spite of this I have made

divisions between samples of some material difference, and by this means graded the samples into ten classes.

This system of classifying offals however cannot be utilised as a method for use outside one particular laboratory since the personal factor is so great. Still in this work it served the useful purpose of seeing how far other methods followed its system of grading.

Physical Composition.

From a physical standpoint wheat offals are composed of moisture, husk, flour, germ and impurities. The percentage of moisture varies somewhat in different samples, and is mainly dependent on the extent the wheat is allowed to dry after washing and before milling, and also on the humidity of the mill. The percentage in the samples I have examined varied between 16·21 and 11·37. If the moisture content rises above 15 per cent. there is a risk of the food turning mouldy very quickly.

The germ of the wheat berry generally finds its way into the offals of medium coarseness (coarse sharps and pollards). Since this constituent is not usually removed during the process of milling the percentage variation is small, and consequently is of little importance in this investigation.

By far the most important of these constituents are husk and flour, and it is therefore apparent that the estimation of these two fractions would go a long way towards solving the problem of grading. Various methods were tried to ascertain the variation in these two constituents.

Sieving.

The separation of the flour from the husk was tried by this method, a "100 mesh" sieve being used. It was found impracticable for the following reasons:

(*a*) During sieving the husk tends to break up especially in a dry sample.

(*b*) A large proportion of the flour adheres to the husk, and can only be partially shaken off even by prolonged sieving.

(*c*) In the finer and whiter offals the flour to a considerable extent exists as "semolina," e.g. in grains of the wheat berry, which have escaped grinding, and consequently do not pass through a fine sieve. These grains are therefore retained with the coarse husky portion.

The only point for which sieving might be utilised is in the separation of the various grades of bran. Here however the difficulty

arises that mills do not all use the same mesh for separating the fine bran from the coarse bran.

Apparent Density.

The densities of husk and flour are widely apart. The following method was evolved which gave figures showing quite a large variation between the finest and coarsest offals.

About 20 grams of the offal were dried in a steam oven at 100° C. for 5 hours (this was found to be ample time for completely drying the substance). After cooling in a desiccator 15 grams were quickly weighed out and transferred to a 100 c.c. graduated cylinder, the cylinder being gently tapped while the offal was sifted in. The cylinder was then jolted on a wooden slab until the contents had reached approximately a constant volume. Finally a 100 gram lead weight (made to fit the cylinder exactly, and having a stout rubber washer glued to the bottom) was gently lowered on to the surface of the offal in the cylinder, and the tapping continued until the volume was constant. The whole test occupied not more than 4 minutes. The results were expressed in c.c.s volume and are given in Table I at the end of this paper.

This test was quite easily and quickly carried out, and repeat determinations made by an independent person gave concordant results. It will be seen from the table that with normal samples this method gave results agreeing fairly well with the classification according to appearance. The following types of samples, however, gave abnormal readings:

(*a*) Samples containing a large quantity of semolina (Nos. 70, 64, 68, 95, 74).

(*b*) Samples of bran which had been rolled, i.e. small bran passed through rollers to make the particles of husk adhere to one another, and thus give the appearance of broad bran.

This test was also used to ascertain the "apparent density" of flour. Five samples were tested, varying from the finest patent flour to a very low grade brand. Three of the samples gave a density of 20 and two a figure of 19·5. I am inclined therefore to assume that this figure (i.e. the apparent density figure) varies directly as the percentages of flour and husk, and the fineness of these two fractions.

Proportion of Flour.

As far as I can see it is only possible to arrive at the proportion of flour in an offal by estimating the percentage of starch in the substance

and multiplying this by a factor. The percentage of starch in flour varies, in normal samples, from 68 to 72 per cent., 70 per cent. being an average figure. Excellent methods are, of course, available for the estimation of starch in flours and similar substances, but my object has been to endeavour to select a speedy and simple method, one which would give approximate but quick results, with a minimum of apparatus and analytical manipulation. The following method, based on one given by Jago in his *Technology of Breadmaking* (p. 814), gave the most successful results.

Five grams of the offal were weighed out and tied up in a piece of fine silk gauze (such as is used in the flour sieves of a roller flour mill). This was immersed in ordinary hard tap water for some time until well soaked, and then kneaded in successive small quantities of water, each washing being poured into a half litre flask. This process was repeated until the wash water showed only a slight opalescence and gave no reaction with iodine solution. The washings were made up to 500 c.c. and 50 c.c. of the well shaken milky liquid were pipetted into a glass tube (a strong test tube was used) and centrifuged in an ordinary hand driven "Gerber centrifuge" for 5 minutes, the maximum speed being about 1400 revolutions per minute. The crude starch was found to be deposited at the bottom of the tube, and the absence of starch in the supernatant liquid could be ascertained by the usual iodine test. The liquid was poured off carefully and more water added, the starch deposit stirred up, and the tube again centrifuged. The deposit was similarly washed with ordinary alcohol and finally with absolute alcohol. After the last washing the deposit of starch was washed into a nickel dish with absolute alcohol, dried at 100° C. and weighed. From this the percentage of crude starch in the offal was calculated.

The crude starch obtained in this way contained a small percentage of protein matter. With fine offals this percentage was fairly constant, 3·8 %, 3·75 %, 3·04 %, and 2·3 % being found in four samples taken. Brans and other coarse offals however gave a crude starch containing rather more protein matter, 13·5 % being the average percentage of several determinations made.

A method was tried by which a known volume of the starch liquid obtained from washing the offal was centrifuged in a graduated tube and the deposit measured. This would have simplified the estimation and saved time, but it was found that starches from offals of different mills (i.e. from different "mixes" of wheat) did not give comparable readings, owing to varying density of the deposit, the factor for converting

the divisions of the tube used into percentages of crude starch varying from ·7 to 1·1. Certainly the majority of the samples tested gave a factor between ·85 and ·95, but even then the calculated percentage would have been very approximate.

Local names of Offals.

The names under which the samples were sent from the different localities are given in Table II at the end of this paper. They are arranged according to the classes specified in Table I.

Purity of Offals.

The samples were all examined for impurities. The chief ones found in wheat offals are weed seeds and oat husk, and these two were the only ones found in the samples examined. More rarely are found barley husk, rice husk, coffee bean husk and tapioca. Reference to Table I will show the purity of each of the samples. 31·5 °/$_{\circ}$ were pure, 53·3 °/$_{\circ}$ moderately pure and 15·2 °/$_{\circ}$ impure.

It is a very debatable question whether the presence of substances occurring in wheat of commerce other than wheat grains should legitimately form part of wheat offals. Wheat of commerce generally contains as impurities weed seeds, dirt, beans and peas, oats, barley and a few minor substances. These the miller is bound to remove as far as possible from the wheat before grinding. Generally the weed seeds are the only impurities which are ground up and returned to the offal, since the other impurities (except the dirt) have a higher market value when sold separately than if ground up and sold with the offal. In my opinion however it is a distinct form of adulteration for a miller to grind up and mix with the pure wheat offal an impurity which during the process of manufacture has of necessity been removed from the wheat. Weed seeds certainly have some feeding value and most of them are harmless substances, but still I consider that the miller should endeavour to put his offals on the market as pure as possible, especially if he wishes these foods to take their place with the other important concentrated feeding stuffs of a farm.

Conclusions.

It is quite evident from the results of this preliminary investigation that the physical composition of milling offals varies between very wide limits. For instance "sharps" may contain anything from 25 to 45 °/$_{\circ}$ of starch, and a "bran" may vary from 10 to 20 °/$_{\circ}$ of starch. Not

only do these variations occur in offals from different mills, but one mill may turn out samples under the same name, but of widely different physical composition.

The names given to offals in different localities are very confusing, and to one unaccustomed to the districts where these names are in vogue, they are of very little use in determining the grade of offal.

The percentage of moisture varies between 11 and 16 %. Those containing more than 15 % are liable to turn mouldy.

With a view to grading milling offals the following determinations should be made:

Percentage of moisture.
Apparent density.
Percentage of starch (or flour).
Purity.

The method given in this paper for the determination of starch is not very satisfactory, a method being required which would give rather more accurate results but at the same time not increasing the time or manipulation required for each estimation. Such a method would take a similar place in flour mills to that occupied by the Gerber test for fat, in dairies.

Recommendations.

1. A series of determinations, such as suggested above, to be agreed upon.

2. A series of grades of offals to be arranged, with stated limits for percentage of starch (or flour) and apparent density.

3. Limits for all offals as regards percentage of moisture and purity.

4. Local names to be dropped as far as possible and such names as "Fourths," "Thirds," "Seconds," "Bran" and "Broad Bran" to be utilised for the standard grades.

5. Millers to be asked to cooperate in some definite scheme for bringing all wheat offals within the range of a system of standardisation such as suggested here.

I wish to express my best thanks to those members of the Agricultural Education Association and to the several millers and agriculturists who so kindly assisted me in this investigation by collecting and forwarding samples.

I must also acknowledge the help of Mr Leonard Ashworth, who carried out many of the analytical determinations.

Table I.

Note. The samples are arranged according to the "appearance" classification, both as regards the grades, and the position of the samples in each grade.

Nomenclature. Column 3. M. = Mixture, E. = English, F. = Foreign.
Column 7. P. = Pure, W.S. = Weed seeds, O.H. = Oat husk, v.s.p. = very small percentage, s.p. = small percentage, m.p. = moderate percentage, l.p. = large percentage.

No. of sample	Name of sample	Wheat from which offal was produced	Moisture per cent.	Apparent density figure	Crude starch per cent.	Purity
			Grade I			
38	Middlings	M.	11·45	26	48·8	W.S. v.s.p.
70	,,	M.	12·06	30	44·8	P.
64	Fine Middlings	E.	14·22	28	44·8	W.S. m.p.
68	,, ,,	M.	11·50	27·5	55·2	W.S. s.p.
			Grade II			
95	Fine Sharps	M.	13·65	31	42·3	P.
33	Fine White Thirds	F.	13·35	25	39·4	P.
74	Boxings	—	13·57	31	40·8	P.
58	Fine Middlings	M.	14·20	28	42·6	W.S. m.p.
			Grade III			
43	Best Fine Sharps	M.	11·82	28	—	W.S. s.p.
17	Thirds	M.	13·45	25	36·8	W.S. m.p.
9	Middlings	M.	13·87	27·5	33·6	W.S. v.s.p.
32	Fine Thirds	F.	12·43	27	36·8	W.S. l.p.
21	Thirds	M.	13·50	31	35·8	W.S. s.p.
92	Straight Run Middlings	M.	13·33	30·5	—	,,
89	Fine Middlings	M.	13·30	28	35·2	,,
94	Sharps	E. (stone ground)	12·78	27	—	W.S. l.p., O.H. l.p., and straw
81	Boxings	M.	16·20	29	36·8	W.S. s.p.
25	Wheat Parings	M.	13·43	27	45·8	W.S. v.s.p.
85	Pollards	M.	12·86	29	—	W.S. m.p.
52	Supers	E.	14·15	30	—	P.
			Grade IV			
69	Fine Sharps	M.	12·24	30		W.S. s.p.
12	Fine Toppings	E.	12·50	32	—	W.S. v.s.p.
41	Fine Sharps	M.	11·77	31	31·2	W.S. m.p.
78	Supers	M.	15·31	31	—	W.S. s.p.
84	Boxings	M.	13·31	33	31·8	W.S. v.s.p.
6	Middlings	M.	12·73	32	—	W.S. m.p.
63	Coarse Middlings	E.	14·40	33	—	W.S. s.p.
37	Sharps	M.	13·05	30·5	31·2	,,
57	Coarse Middlings	M.	14·98	34	30·4	W.S. m.p.
71	Sharps	M.	12·98	30·5	30·8	P.
4	Toppings	E.	12·10	34	—	P.
			Grade V			
1	Sharps	M.	12·40	35	—	W.S. v.s.p.
75	Coarse Pollards	—	14·30	35	27·5	,,
22	Sharps	M.	15·25	33	—	W.S. l.p.
40	,,	M.	11·85	34	28·8	W.S. v.s.p.
50	,,	E.	14·91	34	29·7	,,
88	Coarse Middlings	M.	13·15	35	—	,,

Table I (*cont.*)

No. of sample	Name of sample	Wheat from which offal was produced	Moisture per cent.	Apparent density figure	Crude starch per cent.	Purity
		Grade VI				
80	Sharps	M.	11·37	41	—	W.S. s.p.
30	Common Thirds	F.	13·50	41	—	P.
20	Pollard	M.	14·00	45·5	24·5	W.S. s.p.
49	Pollards	E.	14·17	47	—	W.S. v.s.p.
45	Coarse Sharps	M.	11·57	43	23·2	P.
36	Pollards	M.	14·12	55	—	W.S. s.p.
98	Shorts or Pollards	M.	13·82	40	33·6	W.S. v.s.p.
67	Coarse Sharps	M.	12·57	39	—	P.
10	Pollards	M.	13·70	47	—	P.
16	Pollard	M.	13·55	44	25·2	W.S. v.s.p.
55	,,	M.	15·00	51	—	W.S. m.p.
41	Pollards	M.	11·38	37	25·5	P.
66	Pollard	M.	12·40	53	—	W.S. v.s.p.
61	,,	E.	14·67	51·5	23·2	W.S. s.p.
		Grade VII				
8	Bran	M.	12·90	65	—	P.
99	Common Bran	M.	14·42	49	23·5	W.S. s.p.
26	Medium Bran	M.	13·77	53	—	W.S. v.s.p.
2	Bran	M.	12·46	64	—	W.S. v.s.p.
14	,,	M.	12·90	52	22·7	W.S. s.p.
7	,,	M.	13·07	61	—	W.S. v.s.p.
24	,,	M.	15·03	60	19·3	W.S. m.p.
18	,,	M.	14·90	59	21·2	W.S. s.p.
		Grade VIII				
35	Bran	M.	13·58	66	19·2	P.
11	,,	M.	13·80	63	—	P.
91	,,	M.	13·12	59	—	P.
54	Ordinary Bran	M.	14·54	66	20·2	W.S. v.s.p.
46	,,	M.	12·23	58	17·0	W.S. s.p., O.H. s.p.
83	Bran	M.	13·96	58	18·7	W.S. v.s.p.
73	,,	M.	14·06	69	—	W.S. v.s.p.
86	,,	M.	12·82	58	—	W.S. m.p., O.H. m.p.
48	,,	E.	14·40	64	22·2	P.
65	,,	M.	12·70	68	21·9	O.H. s.p.
28	Broad Red Bran	F.	13·60	69	—	P.
		Grade IX				
77	Bran	M.	14·47	55	—	W.S. v.s.p.
97	Straight Run Bran	M.	13·64	65	21·0	P.
76	Bran	—	14·16	50	—	W.S. v.s.p.
51	,,	E.	14·97	55	—	P.
19	Broad Bran	M.	13·90	75	17·8	W.S. v.s.p.
93	Broad Straight Run Bran	M.	12·98	70	17·8	P.
5	Bran	E.	13·53	70	—	P.
79	,,	M.	16·21	53	—	P.
96	Broad Bran	M.	13·63	67	18·7	P.
27	,,	M.	13·75	69	—	W.S. v.s.p.
87	,,	M.	12·78	63	—	W.S. v.s.p.
42	Bran	M.	12·24	64	16·6	W.S. v.s.p., O.H. m.p.
90	Broad Bran	M.	13·03	68	17·6	P.
60	Ordinary Bran	E.	14·72	67	—	P.
29	Broad White Bran	F.	11·95	50	—	W.S. v.s.p., O.H. v.s.p.
15	Broad Bran	M.	13·25	72	17·8	O.H. v.s.p.
		Grade X				
23	Broad Bran	M.	15·32	78	18·2	P.
53	,,	M.	14·45	83	—	P.
47	,,	M.	12·85	77	14·9	P.
72	,,	M.	13·56	85	14·8	P.
59	,,	E.	15·25	82	—	P.
82	,,	M.	14·00	69	15·2	O.H. v.s.p.

TABLE II.

Grade.........	I	II	III	IV	V	VI	VII	VIII	IX	X
....	Fine Middlings	—	—	Fine Sharps Straight Run Middlings	—	Coarse Sharps Pollards	—	Bran	—	—
....	Fine Middlings	Fine Middlings	—	Coarse Middlings	Sharps Middlings	Pollards	—	Bran	Bran	Broad Bran
....	—	—	Middlings	—	—	Pollards	—	Bran	—	—
....	—	—	—	Toppings Fine Toppings	—	—	—	—	Bran	—
....	—	Fine Middlings	—	—	Coarse Middlings	Pollards	—	—	Bran	—
set	Middlings	—	—	Sharps	—	Pollards	—	Bran	—	Broad Bran
omery	—	—	Sharps	—	—	—	—	—	—	—
....	—	—	—	Middlings Toppings	Sharps	—	Bran Medium Bran	—	—	—
....	—	—	Fine Middlings Straight Run Middlings	—	Coarse Middlings	—	—	Bran	Broad Bran Broad Straight Run Bran	—
k	—	—	Supers	Supers	—	—	—	—	Bran	—
er...	—	—	Fine Sharps	—	—	Pollards Shorts	Common Bran	—	Straight Run Bran Broad Bran	—
ire	—	—	Best Fine Sharps	Ordinary Fine Sharps	Sharps	Coarse Sharps Pollards	—	Ordinary Bran	Bran	Broad Bran
hire	—	—	Thirds	—	—	Coarse Sharps Bran Sharps Pollards	Bran	—	Broad Bran	—
n	—	—	—	Boxings	—	—	—	—	—	Broad Bran
rland	—	—	Pollards	—	—	—	—	Bran	Broad Bran	—
mberland	—	Boxings	Boxings	—	Coarse Pollards	Sharps	—	—	Bran	—
rgh	—	—	Wheat Parings	—	—	—	Medium Bran	—	Broad Bran	—
w	—	Fine Thirds	Fine Thirds	—	—	Common Thirds	—	Broad Red Bran	Broad White Bran	—

THE ESTIMATION OF PHOSPHATES IN SOIL EXTRACTS.

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THE estimation of phosphate in soil extracts presents certain difficulties owing to the small amount usually present. Practically all the methods make use of the phospho-molybdate precipitate. Hehner's method based on the weighing of this precipitate was adopted by Dyer[1], and has been recently investigated by Auld[2].

Of recent years, a more rapid method has been commonly adopted, and is recommended by the U.S. Division of Chemistry[3], in which the phospho-molybdate precipitate is dissolved in standard alkali and the excess of alkali titrated back with standard acid, using phenol phthalein as indicator. The factor in general use for this method is based on the following equation:

$$(NH_4)_6 \,.\, (PO_4)_2 \,.\, 24MoO_3 + 46NaOH$$
$$= 23Na_2MoO_4 + (NH_4)_2MoO_4 + 2\,(NH_4)_2HPO_4,$$

which gives a factor for 1 c.c. $\frac{N}{10}$ alkali = ·0003088 gm. P_2O_5. The method was originally proposed by H. Pemberton[4], who precipitated at a temperature of about 70°. The equation was based on the work of Hundeshagen[5], who found that it held where the yellow precipitate had been dried at 130° or 150° C. The desiccator dried compound was distinctly more acid, and this was attributed to a combination with the

[1] *Journal of the Chemical Society*, 1894, **65**, 115.
[2] *Analyst*, 1912, **37**, 130.
[3] Bulletin 107.
[4] *Journal of the American Chemical Society*, 1893, **15**, 382; 1894, **16**, 278; 1895, **17**, 178.
[5] *Zeits. Anal. Chem.* 1889, **28**, 141.

acid used in the precipitation. To this substance he assigned the formula:

$$12MoO_3 . PO_4 . (NH_4)_3 . 2HNO_3,$$

which would require 25 equivalents of NaOH instead of 23. This brings the factor for 1 c.c. $\frac{N}{10}$ alkali to ·0002842 gm. P_2O_5.

On the other hand, Wolcott Gibbs[1] has given to the yellow precipitate the constitution $48MoO_3 . 2P_2O_5 . 5(NH_4)_2O$, which requires 47 equivalents of NaOH instead of 46 as in the original assumption. This gives a factor for 1 c.c. $\frac{N}{10}$ alkali of ·0003023 gm. P_2O_5.

The author has made a series of experiments to ascertain the best conditions of working this method, and a description of the procedure finally adopted is given on p. 119. These experiments emphasize the necessity of avoiding the use of too high a temperature. Precipitation at 90°—100° gives values which are about 10 °/₀ higher than those obtained at lower temperatures, but the precipitate is apt to be variable in composition and no factor will give correct results. Approximate results could be obtained by using ·00028—·00029, values which fluctuate round ·0002842 based on the constitution assigned by Hundeshagen to the desiccator dried precipitate. From a temperature of 70°, down to room temperatures, the yellow precipitate has a constant composition, and therefore the precipitation should be carried out within this range of temperature and not above it. A temperature of 55° C. is recommended for the precipitation, as this is sufficiently high to bring down the precipitate rapidly and in good condition; a large variation in the temperature in either direction makes no difference to the actual result obtained, although it does to the convenience of working.

The presence of excess of alkali in the final titration results in the production of free ammonia in the liquid, necessitating the rapid manipulation of the titrations. The indicator used—phenol phthalein—is sufficiently sensitive, but owing to the presence of ammonium salts it is necessary to use at least 12 drops of the indicator made up in the usual way.

Pemberton asserts that the presence of silica does not affect the accuracy of this method, but this would appear to be incorrect; experiments described on p. 118 show that the results in presence of silica are too high, probably owing to the precipitation of silico-molybdic acid.

[1] *Journal of the Amer. Acad. of Arts and Science*, 1882, **17**, 62.

Determinations made with solutions containing known amounts of phosphate yielded results 3 °/₀ too high when the calculations were based on Pemberton's factor; apparently therefore the formula

$$(NH_4)_6(PO_4)_2 . 24MoO_3$$

only represents approximately the constitution of the yellow precipitate obtained under the ordinary analytical conditions, and a factor for 1 c.c. $\frac{N}{10}$ alkali of ·0003004 gm. P_2O_5 is therefore recommended. This agrees more nearly with the Wolcott Gibbs constitution.

Experiments to show the degree of accuracy obtained with soil extracts are given on p. 116.

Another method for estimating small quantities of P_2O_5 is that of Brearley and Ibbotson[1], which is used in the analysis of steel and iron for the estimation of phosphorus. In this method the phospho-molybdate precipitate is converted to lead molybdate which is weighed as such. This method, however, was found to be no more accurate than the titration method above described, and it is not so rapid (pp. 117–119).

Experimental Part.

The sodium phosphate used in the following experiments was a pure recrystallised sample. The standard gravimetric analysis by means of magnesium mixture gave the following results: for a solution containing ·5275 gm. $Na_2HPO_4 . 12H_2O$; the $Mg_2P_2O_7$ obtained was:

(1) ·1658 gm. corresponding to ·1057 P_2O_5,
(2) ·1642 gm. corresponding to ·1047 P_2O_5.

The theoretical amount of P_2O_5 being ·1041 gm.

Effect of Temperature on Precipitation. Pure Phosphate Solution.

A solution of sodium phosphate containing ·01135 gm. in 50 c.c. was heated to the temperature desired, with the addition of 10 c.c. of 20 °/₀ sulphuric acid and 25 c.c. of concentrated ammonium nitrate solution (p. 119). 25 c.c. of ammonium molybdate solution (p. 119), previously brought to the same temperature, were then added, and the mixture stirred and allowed to stand for two hours. The precipitate was filtered off, washed with 2 °/₀ sodium nitrate till free from acid, and

[1] *Analysis of Steel-works Materials.*

dissolved in standard alkali. The excess of alkali was titrated back with standard acid, using phenol phthalein as indicator.

The factors in use were based on the theoretical factor

$$1 \text{ c.c. } \frac{N}{10} \text{ alkali} = \cdot 0003088 \text{ gm. } P_2O_5,$$

and the more correct factor ·0003004. The alkali used was not strictly decinormal, and in the following Tables the factors used have been obtained by multiplying the above factors by the necessary correcting factor.

Temp. of precipitation	c.c. alkali	P_2O_5 Theoretical factor ·0003088	$\frac{\text{Actual} \times 100}{\text{found}}$	P_2O_5 Corrected factor ·0003004	$\frac{\text{Actual} \times 100}{\text{found}}$
90—100°	39·7	·01253 gm.	—	·01219 gm.	107·4
	39·65	·01251	—	·01217	107·3
55°	36·75	·01160	102·2	·01128	99·4
	36·9	·01164	102·6	·01133	99·9
Room temp.	36·5	·01152	101·5	·01121	98·8
	37·05	·01169	103 0	·01137	100·2

The alkali used was 1·023 decinormal; the factors actually used were therefore ·0003088 × 1·023, ·0003004 × 1·023, i.e. ·000316 and ·000307 respectively.

It thus appears that precipitation at a temperature of 55° C. gives the best results, while the factor ·0003004 is the nearest one can use. This was confirmed in the following experiments.

A solution containing ·01022 gm. P_2O_5 in 50 c.c. (a pure sodium phosphate) was precipitated at 55° C. as above.

Volume of alkali, c.c.	P_2O_5 indicated (gms.). Factors:		Ratio $\frac{\text{Found}}{\text{Actual}} \times 100$. Factors:	
	(1) ·0003088*	(2) ·003004*	(1) ·0003088	(2) ·003004
1. 33·7	·01049	·01021	102·3	99·8
2. 33·9	·01055	·01027	103·2	100·4
3. 33·85	·01053	·01025	103·0	100·2

* For the alkali used ·0003113 and ·0003028 respectively.

Soil Extract (Effect of Temperature on Precipitation).

100 c.c. of a citric acid extract were evaporated to dryness, and the residue ignited to remove iron, organic matter and silica. The residue was taken up by digestion with 10 °/ₒ sulphuric acid and the extract precipitated with ammonium molybdate as above.

That the extraction with 10 °/ₒ sulphuric acid is efficient was proved by extracting the residues with aqua regia, evaporating to remove the HCl, and taking up with HNO_3, then adding ammonium nitrate and ammonium molybdate; in no case was any phosphate recovered.

100 c.c. Soil Extract; Factor for $\frac{N}{10}$ alkali 1 c.c. = ·0003004 gm. P_2O_5.*

Temp. of precipitation	c.c. alkali	P_2O_5 indicated gms per 100 c.c.
90—100°	38·9	·01173
	39·0	·01176
55°	35·2	·01061
	35·5	·01070
Room temperature (stand 24 hours)	35·25	·01063
	35·45	·01069

$$\text{Ratio } \frac{\text{found at } 100^\circ}{\text{found at } 55^\circ} \times 100 = 110{\cdot}1.$$

* For the alkali used ·0003016.

The experiment shows that precipitation at 100° gives results 10 °/ₒ higher than precipitation at 55°, just as it did when pure sodium phosphate was used.

Effect of Silica on the Precipitation.

It was necessary to investigate this point because of the presence of silica in extracts obtained when soil is treated with citric or nitric acids.

To a solution of sodium phosphate a solution of colloidal silica, free from phosphate, was added, and the precipitation carried on as above.

The filtrates showed a characteristic yellow colour.

Thus in presence of silica the results come out rather too high, and steps therefore have to be taken to remove all silica before precipitation with the molybdate.

Solution of sodium phosphate = ·01122 *gm.* P_2O_5.

1 *c.c.* $\frac{N}{10}$ *alkali* ·0003004 *gm.* P_2O_5*.

c.c. alkali	P_2O_5 indicated	Ratio $\frac{\text{Found}}{\text{Actual}} \times 100$
No silica present		
1. 36·5	·01120 gm.	99·8
2. 36·65	·01124	100·2
3. 36·6	·01123	100·1
Silica present		
1. 37·8	·01160	103·4
2. 37·7	·01157	103·1
3. 37·75	·01158	103·2

* For actual alkali used ·000307.

Application to Soil Extracts.

In order to test the applicability of the method to soil extracts a known amount of sodium phosphate was added to an extract, and the phosphate determined before and after the addition. Although the experimental error is somewhat greater in this case, the results show that the method can be relied on to give accurate values in the case of soil extracts prepared as described on p. 120.

Soil extract A (*an HCl soil extract*).

100 c.c. A	Vol. of alkali (factor ·0003004)*	P_2O_5
1	20·6 c.c.	·006324 gm.
2	20·7	·006355
	Mean	·006339
100 c.c. A + 25 c.c. of a phosphate solution (= ·00561 gm. P_2O_5)		
3	38·8	·011912
4	38·85	·011927
	Mean......	·011920

* For actual alkali ·000307.

Amount of P_2O_5 indicated = ·00558 gm. (·011920 − ·006339).
Actually added = ·00561 gm.
Ratio $\frac{\text{Found}}{\text{Actual}} \times 100 = 99·4$.

Soil extract B.

50 c.c. B	Vol. of alkali (factor ·0003004)*	P_2O_5
1	30·9 c.c.	·009436 gm.
2	30·85	·009471
3	30·6	·009394
	Mean......	·009447

* For alkali used ·000307.

25 c.c. B + 25 c.c. of a phosphate solution (= ·005925 gm. P_2O_5).

	c.c. alkali	P_2O_5
1	34·6	·010622 gm.
2	34·4	·010561
3	34·7	·010653
4	34·4	·010561
	Mean.....	·010599

25 c.c. B = ·004723 gm. P_2O_5.
*Amount added (as found) ·005876 gm. (·010599 – ·004723).
Actually added ·005925 gm.

$$* \text{ Ratio } \frac{\text{Found}}{\text{Actual}} \times 100 = 99{\cdot}1.$$

Lead Molybdate Methods.

Brearley and Ibbotson adopt two methods. In the first the phospho-molybdate is dissolved in dilute ammonia, and sufficient HCl is added to prevent the reformation of the yellow precipitate. A sufficient amount of lead acetate is now added and ammonium chloride, and sufficient ammonium acetate to neutralise the free HCl—all at boiling temperature. The lead molybdate comes out as a white precipitate, while a soluble acid lead phosphate is said to remain in solution. The P_2O_5 is calculated from the ratio $P_2O_5 : 24PbMoO_4$.

In the second method, the ammoniacal phospho-molybdate solution is made acid with acetic acid and then precipitated with lead acetate at boiling temperature; a mixture of lead phosphate and lead molybdate is said to be obtained, and the P_2O_5 is calculated from the formula $Pb_3(PO_4)_2 + 24PbMoO_4$. The precipitates in both cases are filtered off through an ordinary filter, washed several times with hot water, and ignited in a muffle or over a Bunsen burner.

In repeating these methods with pure phosphate solutions it was found that the first method gave slightly high results, while the second gave results that were much too low, if calculated on the above assumption. As in both methods the end condition of the solution is practically the same, except for the large amount of ammonium chloride present in the first method; it is difficult to see why the lead phosphate should be held up in one and not in the other. Lead phosphate is soluble to a certain extent in acetic acid, and a modification of the second method has therefore been tried, in which the final precipitate is washed several times with hot dilute acetic acid to remove the lead phosphate. Results slightly too high have been obtained, but which compare favourably with those obtained by Brearley and Ibbotson's first method.

Experimental.

Method 1. A solution of sodium phosphate containing 0·00484 gm. P_2O_5 was precipitated with ammonium molybdate at 55° C. as in the first part of this paper. The phospho-molybdate precipitate was filtered off, washed with dilute nitric acid, and dissolved in 4 % ammonia. The ammoniacal solution was acidified with 100 c.c. concentrated HCl, 10 c.c. of a solution of lead acetate (40 gms. to 1 litre) were added, and the whole brought to boiling point. 50 c.c. of ammonium chloride solution (200 gms. to the litre), and 50 c.c. of concentrated ammonium acetate were heated together and poured into the first solution. The precipitate was filtered off and washed several times with hot water till all traces of chloride were removed. The precipitate was ignited in a porcelain crucible over a Bunsen burner.

Wt. of $PbMoO_4$	P_2O_5 (factor ·01613) Found	Actual	Ratio $\frac{\text{Found}}{\text{Actual}} \times 100$
1. ·3078 gm.	·004965	·00484	102·6
2. ·3040	·004905	·00484	101·4

Method 2. The solution of ammoniacal phospho-molybdate was acidified with 10 c.c. acetic acid and brought to boiling point. To it were added 10 c.c. of the above lead acetate solution—the precipitate was filtered off, washed with hot water, and ignited as above.

A. Calculated as lead phosphate + lead molybdate (factor ·01477).

Wt. of precipitate	P_2O_5 (gms.)		Ratio $\frac{\text{Found}}{\text{Actual}} \times 100$
	Found	Actual	
1. ·3145 gm.	·004644	·00484	95·96
2. ·3112	·004596	·00484	94·97

B. Calculated as lead molybdate (factor ·01613).

Wt. of precipitate	P_2O_5 (gms.)		Ratio $\frac{\text{Found}}{\text{Actual}} \times 100$
	Found	Actual	
1. —	0·005072	—	104·8
2. —	0·005020	—	103·7

It is clear that in A the whole of the phosphoric acid has not been thrown down with the lead molybdate, as supposed by Brearley and Ibbotson.

Method 3. The solutions were precipitated as in Method 2, and the precipitate washed several times by decantation with hot dilute acetic acid (about 5 °/$_o$).

The precipitate was assumed to be $PbMoO_4$.

Wt. of precipitate	P_2O_5 (gms.)		Ratio $\frac{\text{Found}}{\text{Actual}} \times 100$
	Found	Actual	
1. ·3224 gm.	·005200	·005112	101·7
2. ·3257	·005253	·005112	102·8

Method finally adopted.

Reagents wanted:

1. Concentrated ammonium nitrate, 500 gms. of ammonium nitrate, 1 litre of water.

2. Ammonium molybdate solution, 150 gms. ammonium molybdate dissolved in 1000 c.c. of water and poured into 1000 c.c. of nitric acid (S.G. 1·2).

3. 2 °/$_o$ sodium nitrate.

Preparation of Soil Extracts.

A measured volume of soil extract containing 5 to 10 mgs. P_2O_5 is evaporated to dryness on a gently heated sand bath, and the residue ignited at a dull red heat for 15 minutes as in Neubauer's[1] method. The residue is taken up with 50 c.c. of 10 % sulphuric acid and digested for half an hour on a sand bath. The extract is diluted, if necessary, filtered, and the residue washed with hot water; filtrate and washings amounted to 110 c.c. This procedure is found to extract all the phosphate, when the amount of the original solution is not more than 100 c.c. in the case of an HCl extract. For soil extracts containing much silica[2] it is necessary to heat the residue from the evaporation for two hours at 120° to 160°, the silica interfering otherwise with the subsequent manipulations.

Precipitation of the Phospho-molybdate.

To the solution prepared as above 25 c.c. of the concentrated ammonium nitrate is added and the mixture brought to 55° C. 25 c.c. of the ammonium molybdate, previously brought to the same temperature, is then added and the mixture stirred, allowed to cool, and filtered after standing two hours.

The supernatant liquid is decanted through a filter paper, and the precipitate washed by decantation several times with a 2 % sodium nitrate solution; this solution prevents the deflocculation of the precipitate, which usually happens when distilled water is used alone. The washing is continued till the washings are no longer acid. The filter is then washed into the beaker with water, and the precipitate dissolved in standard alkali and titrated back as stated previously. For the precipitation it is found convenient to use a water bath kept at 55°, in which the beakers containing the solution are placed till they have acquired the temperature of the bath. The factor recommended for $\frac{N}{10}$ alkali is 1 c.c. = ·0003004 gm. P_2O_5.

[1] *Landw. Vers. St.* 1905, **63**, 141.

[2] Extracts obtained by shaking soil with dilute acid solvents such as 1 % citric acid contain considerable quantities of silica.

ERIOPHYES RIBIS (NAL.) ON RIBES NIGRUM.

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THE life history of the black currant mite, *Eriophyes ribis*, Nal. is of a simple character from the month of May, when the buds of the current year's growth are entered, till March of the following year. During this time the mites live and multiply in the embryonic leaves producing the abnormal growth familiar to fruit growers as "big-bud." From the end of March onward, however, the buds so attacked gradually die, and the mites which have lived in them for the past nine months are forced to find other plant food. At this time the buds of the black currant which the mites will eventually live in are rudimentary and it is the end of May before they are sufficiently developed for the acarids to enter.

An interval of two months therefore occurs between the time when migration becomes necessary in March, to the time when mites are found in the new buds in May.

How the mites exist during this time has been the subject of conjecture and this question and the mode of migration have been dealt with in this investigation.

When the attacked buds of the black currant tree are examined in March, April and May, it is seen that, according to certain weather conditions, more or less active migration of the mites is taking place. They emerge from between the leaves of the deformed bud and distribute themselves over its outer surface. If the temperature is low, there is no apparent purpose in their movements and they crawl over the scale leaves in a lethargic manner and return to the interior of the bud again. If, on the other hand, weather conditions are favourable, *i.e.* temperature high, sun shining and a breeze blowing, the mites become extremely active and with a high powered lens this critical period in their life history may be followed.

A few mites then leave the buds and make their way down the stem, a few make a sudden spring and disappear from sight and presumably alight on the leaves beneath the bud, but by far the greatest number crawl actively about the bud and then suddenly stand erect.

This curious habit was first recorded by Warburton[1], and it appears from these observations that under favourable conditions at least 80 °/₀ of the mites act in this way on leaving the interior of the bud.

That this habit plays a most important part in the distribution of the species can be demonstrated in the following manner.

If a big-bud from which migration is taking place is covered with a glass tube on a windy day when the temperature is high, it is seen through the glass that hundreds of mites have come from the interior of the bud and that, with the exception of a few, all are standing erect on the muscular disc present at the posterior end of the abdomen. If the glass is then removed and the mites so standing are exposed to the wind, they are carried away instantly by it, until a few only remain of the hundreds that were present on the surface of the bud. Although it is usual for the acarids to be blown away as they stand erect in the wind, yet they appear to have control over their movements when in this position, for they can be seen to sway in the breeze and yet remain firmly fixed to the bud. On a calm day mites may be seen standing erect for several minutes apparently waiting for the wind to carry them away.

Distribution may thus be said, in the case of the mite on the black currant, to be effected mainly by the agency of the wind.

There are three other methods by which the mites are distributed at this period:

(i) By clinging to passing insects, such as bees, thrips, etc. and being carried by them to surrounding trees.

(ii) By being carried up with the developing shoot, in the event of the "big-bud" surviving the attack, or in the case of the bud being killed by crawling to surrounding shoots.

(iii) By springing from the "big-bud" to other parts of the tree.

The two last named methods would ensure re-infection of the attacked tree.

The question of the relative degree of activity of the mites at a high and low temperature has already been mentioned.

It is found that the day temperature during the migratory period

[1] *Annual Report of the Zoologist*, Warburton, *R.A.S.E. Journal*, 1901.

varies roughly from 50° to 100° F. Intervals of hot sun send the thermometer up to the latter temperature and it is these hot spells which bring the mites swarming to the surface of the buds. On such days the wind is often intermittent and gusty and admirably suited to carrying the mites to long distances.

At the extremes mentioned there is marked difference in the number of mites which come from the big-buds and in their subsequent behaviour. At 50° F. few mites come from interior of the buds and they make no attempt to raise themselves to an erect position. When the thermometer rises above 80° however the mites become extremely active and the erect position is generally adopted.

The following field experiment, the results of which I do not consider in any way conclusive, for the number of buds examined was too small to give an accurate record, was undertaken, firstly with the object of finding out the number of mites which emerges from the buds at given temperatures, and secondly of roughly estimating the number of mites distributed by the wind under the same conditions.

TABLE I.

Date	Temp. 50° F.		Temp. 60° F.		Temp. 65° F.		Temp. 70—80° F.		Temp. 80—90° F.		Temp. 90—100° F.	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
May—June	6	6	40	18	48	15	77	49	94	15	106	3
	1	1	34	16	52	20	83	25	111	23	83	4
	2	2	61	32	63	26	75	10	75	11	82	2
	3	3	53	29	73	49	82	45	93	9	101	—
	2	2	46	32	49	17	81	42	102	5	141	5
	14	14	234	127	285	127	398	171	475	63	513	14

A. No. of mites which emerged from buds.
B. ,, ,, ,, remained on bud after exposure to wind.

For this purpose big-buds from which active migration was taking place were brushed with a camel hair brush to remove any acarids which might be on them, and screened from the wind for a period of five minutes. The temperature was recorded from a thermometer hanging on the tree under observation. After the five minute period had elapsed, the number of mites which had emerged from the buds was counted. The buds were then exposed to the wind for one minute.

From a reading of the mites which remained on the buds after exposure to wind, it was possible to roughly estimate the number of mites which had been distributed by this means.

On examining the results (Table I), it is seen that at the extremes of temperature there is a marked difference in the number of mites which come from the buds and that in spite of considerable variation in the figures obtained, the aggregate number of mites which emerges between these temperatures follows an ascending scale with a rising thermometer. Also that the number of mites dispersed by wind increases under the same conditions.

By averaging the numbers in each of the five minute periods it is found that each bud may set free under favourable conditions roughly 1000 mites an hour. When this figure is multiplied by the number of "big-buds" (usually running into many hundreds) present on an affected tree, it is obvious that one bush alone may be a source of infection to all orchards in its vicinity.

It will also be seen that the sensitiveness of the mites to the variations of temperature will control their actions during the migratory period, so that in a cold late spring a late exodus of mites will take place and the reverse in an early season.

Another factor which appears to control the number of mites emerging from big-buds at the migratory period is the condition of the bud from which migration is taking place. Some buds and the mites within them die before conditions are suitable for migration, and others, although green and succulent, remain so tightly closed that the mites within are imprisoned.

"Big-buds" are found in all stages of decay from March till the middle of June, and it would seem from the following laboratory experiment, carried out on a limited number of buds, that the number of mites migrating from them depends largely on the degree of succulence of the buds.

For the experiment shoots with big-buds in all conditions, from green to those which were dead, were placed in different temperatures. The green buds were either nearly closed or expanding to such an extent that the mites could crawl readily from all parts of the bud. Before the buds were inserted into the required temperatures, all surfaces were brushed to remove mites already on them. When the buds had been exposed to the various temperatures for a period of ten minutes, they were examined and the number of mites found on their surfaces counted. The following figures were obtained:

No. of buds	Date	Condition of bud	Time exposed	50° F.	60° F.	80° F.	100° F.
5	May 17th	Green, closed	10 mins.	6	38	136	220
,,	,,	,, expanding	,,	16	142	482	906
,,	,,	Becoming dry	,,	5	100	625	1300
,,	,,	Nearly dry	,,	10	35	95	180
,,	,,	Quite dry	,,	—	—	—	—

It will be seen from this experiment that no migration had taken place from buds which were dry, those which were nearly dry and those which were closed gave approximately the same number of emerging mites, while from the green expanding buds and those which were becoming dry the most active migration had taken place. Also that the figures give the same results as the experiment dealing with the influence of temperature on migrating mites.

The following factors may be said to influence the action of the mites at the migratory period.

(i) The wind, which is the principal distributing agent.

(ii) The temperature, which apparently influences the number of mites which comes from the bud, and which stimulates them to adopt the position necessary for successful distribution.

(iii) The condition of the bud at the time when migration is taking place.

The next question to be considered is that of the temporary resting place of the mites during the interval of two months that elapses between the time when migration begins in March till the time when the new buds are entered in May.

It has been suggested that the mites might find shelter under the loose bark that is shed annually by the black currant. Mites of the genus Phyllocoptes were found there, but not *Eriophyes ribis.*

Another suggestion,—that the mites live in the soil during this period—has been put forward.

The surface of the earth round the trees was examined with a lens and also microscopically in the laboratory. Although a few mites were found, they were so occasional and in such apparent difficulties that such surroundings were considered unnatural to them.

Young shoots and leaves were kept under observation and it was found possible with the use of a strong lens to see mites actually blown on to them. When their movements were followed it was found that eventually they made their way, mostly by the veins which are smooth and over which they made rapid progress, to the petiole.

The movements of the mites could be traced until they arrived at the base of the petiole when they disappeared between its upper surface and the main stem where the bud eventually shows itself.

When such a leaf base is dissected, it is found that a small colony of mites has established itself in this position. It is, then, on these succulent inner surfaces of the leaf bases that the mites feed and reproduce, until the buds which are almost invisible are large enough to be entered.

As the season advances, the tissues of the lower leaves of the young shoots harden. This causes most of the mites to leave those leaf bases which no longer give them nourishment, and to migrate to those nearer the apex of the growing shoot. This tendency to seek the young leaves of the shoot where the tissues are soft and succulent is general, so that when a half grown shoot is dissected it is seen that in the lower leaf bases there are many cast skins and but few living mites, while towards the apex, in the leaf bases and in between the young leaves near the growing point, living mites are numerous.

The mites also creep in between the minute bud and the main stem and it is in this position that they are usually found when present in the older leaf bases of the shoots.

The following figures were obtained from the examination of a shoot three inches in length in May:

1st	leaf	from base of shoot......	no cast	skins,	3	mites	between bud and stem
2nd	,,	,, ,,	,,	,,	2	,,	
3rd	,,	,, ,,	,,	,,	5	,,	in leaf bases
4th	,,	,, ,,	few	,,	10	,,	,, ,,
5th	,,	,, ,,	,,	,,	19	,,	,, ,,

In the terminal leaflets at apex of shoot were:—

5	mites	crawling over minute terminal bud	
28	,,	,, between folded terminal leaflet	3 mm. in length
33	,,	,, ,, ,, ,, ,,	4 ,, ,,

After an examination of numerous shoots it was found that generally the number of mites in the terminal leaflets exceeded several times the total of those found in the leaf bases.

Attention has been drawn to the fact that the terminal buds of an attacked tree are generally "big-buds." The habit of the mites seeking the apical leaves and buds is probably the reason why this occurs.

An examination of the bud scales which persist for some considerable time round the bases of the new shoots shows that the mites take

shelter there—they also appear to collect in the leaf scars and probably feed on the oil glands which are present there.

At the end of May, the buds of the current year's growth are entered by the mites and it is within them that the life history of the acarids is continued until the migratory period recurs in the spring of the following year.

The following experiment dealing with the infection of trees in the orchard was undertaken to find out:

(i) To what extent new shoots are infected by mites being carried by the wind.

(ii) To what extent infection occurs from a "big-bud" to the shoot growing from it.

(i) *Infection by mites carried by the wind.* This experiment could not be carried out for it was found impossible to get uninfected stock. Bushes which were apparently normal, shewed the presence of mites when the buds were dissected.

(ii) *Infection by mites carried up with the growing shoot.* For this experiment "big-buds" which appeared likely to develop into shoots were taken and pruned directly above the buds. A ring of vaseline was put round the stems immediately below the buds to exclude upwardly crawling mites and insects, and the remaining buds on the branches were removed in order to concentrate the sap in the shoots that would grow from them.

The "big-buds" so treated were then enclosed in waxed muslin bags and the stems below the bags greased again as an additional protection against insects.

Shoots developing from "big-buds" so treated could be infected only by the mites which were carried up with the buds as they expanded since they would be protected from migrants distributed by wind and from mites and insects crawling up the stem.

Five hundred buds were examined from shoots so treated from the middle of May, when the buds were large enough to be dissected, till the middle of June when migration had ceased.

The same number was examined from unprotected shoots and the following unexpected figures were obtained:

Treatment of buds	No. buds	No. mites present in Leaf bases	Terminal leaves etc.	Buds	Total mites	Eggs
i. Greased and covered...........	500	189	353	406	948	38
ii. Ungreased and not covered ..	500	126	131	292	549	25

It is seen that the protected buds contained, contrary to expectation, nearly twice the number of mites found in those left unprotected.

Freedom from parasites and predatory insects would account to a limited extent for the increase of mites in the shoots enclosed in bags.

The small number of mites present in the untreated shoots appeared, however, to be due to some disease, possibly bacterial, which attacked mites in all stages of growth and frequently exterminated the whole colony.

It was thought that severe frosts which occurred during the experiment might have caused the mites exposed to sudden lowering of temperature to die, but experiments carried out in the laboratory shewed that this was improbable. Mites were exposed to a temperature of 15°—20° F. during the day for three successive days. When examined at the end of that time they were still alive, and the same mites when the thermometer was subsequently lowered to zero for several hours, were found to be feebly moving after the temperature had again been raised to normal.

It was evident, from an examination of the old "big-buds" taken from the trees experimented on that the parasitic fungus, *Botrytis eriophyes*, had also caused the death of a large number of mites. Agar plates were inoculated with the fungus taken from such buds and a pure culture was eventually obtained.

The difficulties of working at the life history of a pest to get any accurate knowledge are apparent. Natural checks such as have been described cannot be eliminated and experiments carried out with material kept under abnormal conditions are unsatisfactory.

Nevertheless the experiments described here, conducted for the most part under natural conditions, add something to our knowledge of the manner in which this pest is distributed.

ERIOPHYES RIBIS (NAL.) ON RIBES GROSSULARIA.

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Last season (1912) it was noticed that gooseberry trees were being seriously deformed by an attack of Eriophyes. Specimens of the mite were sent to Dr Nalepa and they were identified by him as *Eriophyes ribis*, the mite responsible for the disease known as "big-bud" in black currants.

Rostrup (1) includes the gooseberry in the list of hosts of *Eriophyes ribis*, and Collinge (2) reports finding mites in the buds of this tree.

Theobald (3) has also recorded an attack of the same mite on the red currant. No proof, however, has yet been given that the species can pass directly from one host plant to another.

Before entering into biological details of the mite on the gooseberry, it will be convenient to summarize the principal points of difference between the habits of the mite on the host plants *Ribes nigrum* and *Ribes grossularia*.

(i) *Ribes nigrum* when attacked by *Eriophyes ribis* developes abnormal buds, known as "big-buds." All parts of the bud, with the exception of the outer scale leaves, are injured.

No phenomenal growth of the buds of *Ribes grossularia* takes place when attacked. The true leaves remain uninjured and the scales only are deformed.

(ii) The tissues of the expanded leaves and shoots of *Ribes nigrum* attacked by the mite shew no sign of injury while in the case of *Ribes grossularia* they are severely blistered and deformed.

(iii) Migration by the agency of the wind is the general method of distribution employed by the mite on *Ribes nigrum*. The same habit is observed to a limited extent on *Ribes grossularia*, the general method being that of crawling from the scale leaves of the attacked bud to the shoot developing from it.

The first question considered in the above summary is that of the production of the big-bud in *Ribes nigrum* and the normal bud in *Ribes grossularia*: and the relative amount of injury done to both hosts.

It has been stated that all parts of the black currant bud with the exception of the outer scale leaves are attacked by the mite. Thus, the future shoot and therefore the vital and essential parts of the bud are injured and the growth of the tree is in consequence severely checked.

An examination of the attacked bud of the gooseberry shews that no mites are present in the true leaves of the bud, but that they have collected on the succulent portions of the scale leaves which surround them.

These scale leaves differ in structure according to the position they occupy. Those which immediately enfold the true leaves are green and succulent, except, perhaps, at the extreme apex, those external to them are partially woody and partially succulent—woody where they are exposed to light and air and succulent where they are covered with other scale leaves. Those present at the base of the bud are hard and woody throughout.

It is on the succulent portions of the leaves that the mites are found from the time when the buds are entered in early summer, until the spring of the following year. Large blisters, which are characteristic of an attack by the mite on this host, are formed on the surface of the scale leaves. These, when they have served their purpose of protecting the embryonic shoot they enclose, fall off.

The nature of the injury is therefore not permanent and the bud, because the mites do not enter the true leaves to deform them, remains normal.

In section II of the summary it is stated that no apparent deformity occurs to the developing leaves and shoots of an attacked black currant tree.

The mites on the gooseberry, when the buds expand, crawl from the scale leaves into the young developing shoot and start puncturing the delicate tissues of the still folded leaflets, the petioles and the main axis. As a result of this continuous suction, these parts when they expand have a striking appearance. On both upper and under surfaces of the leaves, large, raised and often confluent blisters which are turgid with cell sap are seen. The petioles of the leaves and the young main axis shew the same malformations. All parts of the fruit blossom are deformed in the same way and fruit in consequence fails to set.

When the leaves expand the mites still continue to perforate the tissues, until, after a bad attack, the whole leaf surface, the veins and the petiole become covered with succulent outgrowths.

These excrescences, besides giving a plentiful supply of food material, afford the mites a means of protection and the acarids take up their position between them. The bases of the ribs of the leaves especially are surrounded by a mass of these outgrowths and beneath them is found a colony of mites, eggs and nymphs.

Leaves so attacked are considerably under their normal size and the main stem itself is reduced in length. The leaf colour is yellow green, and the tree has a generally unhealthy appearance.

As the season advances the leaves harden, and these excrescences lose their watery contents and become dry and discoloured. The injured epidermis frequently cracks and in severe cases the leaves fall from the tree. The injured bark splits in ribbons and comes away from the stem.

Practically all the injury done to the vegetative portions of the gooseberry occurs in the spring and early summer when the trees are making active growth and the leaf tissues are tender and succulent. Later in the season the texture of the leaves becomes tough, new growth is made slowly and hardens quickly and the mites find a difficulty in getting their food supply.

Therefore, when the buds of the current year's growth are large enough for the mites to enter, they are found to collect in the scale leaves and it is in this position that their life history is completed.

After the mites have entered the buds, the leaves are no longer attacked, and in consequence a marked difference in the size and colour of the subsequent growth is seen. Thus, the leaves which were produced early in the season are small and misshapen, while those of the later growth are normal in size and colour (Fig. 1).

In section iii of the summary it has been stated that differences occur in the habits of the mites at the migratory period.

It has been shown that the mites attacking the black currant emerge from the "big-bud" by hundreds and distribute themselves on the outer surfaces of the scale leaves from which position they are distributed by the wind.

The mites attacking the gooseberry do not collect in numbers outside the buds in this way. When the buds begin to expand, the acarids leave the leaf bases and crawl between the young leaves of the developing shoot.

Mites are occasionally to be seen being distributed from the surface of the leaves by the wind, but the general method of distribution is that already described.

The probable reason for this divergence in the habits of the mites at this period is that a large proportion of the buds of an attacked black currant tree is killed. The mites which have destroyed these buds are therefore forced to adopt some means by which they can come in contact with new plant food.

The buds of the gooseberry on the other hand are not killed and the shoots normally develop from them. The mites consequently have their plant food at hand, and it is unnecessary for them to adopt a uniform migratory action.

FIG. 1.

It is interesting to compare the number of mites present on the two host plants at different stages of their life history.

If attacked buds of the two species of Ribes are examined before migration has taken place in the spring, the number of mites present in the black currant is countless, while a few hundreds only are to be found in the scale leaves of the gooseberry.

When however the foliage of the young shoots of both hosts is examined a few weeks after the buds have expanded, it is found that the mites on the gooseberry have increased so that they are almost countless, while a few mites only can be found in the leaf bases of the black currant.

Nor does the mite on the black currant appear to be so numerically strong as the mite on the gooseberry until the new buds are entered, when a phenomenal increase takes place.

The mite on the gooseberry on the other hand appears to receive a check to reproduction when the buds are entered.

It is possible that the abundant food supply contained in the swollen embryonic leaves of the black currant bud may increase the vigour of the mites and stimulate reproduction, while the scales of the gooseberry, functioning only as protective organs, may not contain the necessary nourishment for rapid reproduction.

It is noticeable that when the mites on the gooseberry commence feeding on the expanding true leaves that rapid increase takes place.

The check to the increase of the mite on the black currant at the migratory period is undoubtedly due to the excessive loss of life caused by the hazardous method of distribution, whilst the method employed by the mite on the gooseberry ensures the safe arrival of most of the mites, and reproduction proceeds without a check.

These observations bring to a conclusion the life histories of the mite *Eriophyes ribis* on its hosts *Ribes nigrum* and *Ribes grossularia.*

It will be of interest to continue the comparison to the trees of the two species of Ribes and examine the structure of the buds which differ under the attack of the mite.

It has been shown that the mites on the gooseberry form excrescences on the scale leaves of the bud and on the vegetative parts of the tree, and it is therefore clear that if the mites could reach the true leaves of the bud they would deform them in like manner. It is therefore probable that there are causes which inhibit the entry of the mites into the centre of the gooseberry bud.

When the buds of the two trees are compared it is seen that there are well-defined morphological differences in the structure of the scale leaves, and it is suggested that these, together with certain other differences, are the probable causes which control the entry of the mites into the buds.

The dissection of a gooseberry bud shews that each scale leaf covers the inner succeeding scale leaf for two thirds of the entire length of the bud. At the base of the bud the margins of the scales are slightly separate, but from the point of their contact upwards, they commence to fold over one another, until at the apex they overlap to the extent of nearly twice encircling the bud. The shape of such leaves when removed from the bud is conical and convolute.

No such overlapping of the scale leaves takes place in the case of the black currant. The margins of the leaves meet at the apex of the bud only, and consequently at the base of the bud nearly half of the inner succeeding leaf is left exposed.

Again, the scale leaves of the gooseberry have at their margins a dense growth of long hairs, while those of the black currant have a sparse growth of short hairs.

The general tightness of the gooseberry bud is very noticeable and contrasts with the lax habit of the black currant bud.

It is probable that these differences in the scales of the gooseberry, *i.e.* overlapping of the leaves, the dense fringe of hairs at their margins and the general tightness with which the leaves fit together combine to form a barrier through which it is impossible for the mites to pass.

The degree of tightness or laxness of the respective buds is partly due to the differences between the epidermis of the scale leaves. The epidermis of the gooseberry scale leaves is smooth and occasionally sparsely covered with hairs, which, however, do not prevent the close contact of the leaves. The scales of the black currant are densely covered, except at their margins, with the oil glands characteristic of this species of Ribes. Between these glands is a dense growth of stiff short hairs.

It is suggested that the presence of these glands and hairs on the surface of the black currant scales prevents their close contact, and permits of the entrance of the mites to the true leaves between the spaces thus formed, and that the absence of such excrescences in the case of the gooseberry, allows the scale leaves to fit together in such a manner as to inhibit the entry of the mites to the interior of the bud.

Again, relatively deep depressions and irregularities occur on the surface of the scale leaves of the black currant bud. If the margins of a scale leaf cover such depressions on the inner scale leaf a considerable space is left between the two leaves. The hiatus thus formed would allow the mites to work their way through the successive scales into the centre of the bud. Such depressions do not occur in the gooseberry bud.

Mites have been frequently seen crawling between the scale leaves of a black currant bud and it is a common occurrence to find the first migrants in the centre of a new bud.

On the other hand mites have not yet been found in the true leaves of a gooseberry bud and but rarely in the inner sheathing scale leaves.

The bud of the red currant, *Ribes rubrum*, is almost identical in

structure with that of the gooseberry and no record has been made of abnormal growth of buds of attacked trees. This would tend to strengthen the supposition that the condition of the bud under attack is determined by the structure and habit of the buds.

It is of interest to notice in what degree the mite affects its different hosts.

Ribes rubrum appears to be the least injured, *Ribes grossularia* certainly has its growth checked early in the season, but recovers from the attack later, while *Ribes nigrum* is so badly crippled after a serious attack that it is of little use to the fruit grower. It is true that the trees keep alive for years, but the yield of fruit is so low that from a commercial point of view it is better to restock a plantation so attacked by a more profitable crop.

The distribution of the mite on *Ribes grossularia* appears to be as universal as that on *R. nigrum*, and most orchards are infected although symptoms of attack are not always conspicuous.

If the gooseberry and red currant should prove capable of harbouring the same mite which produces the big bud disease of the black currant it will be necessary to adopt preventive measures on these three species of Ribes if the pest is to be kept under control.

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THE EFFECT OF ONE CROP UPON ANOTHER.

By THE DUKE OF BEDFORD, K.G., F.R.S.

AND

SPENCER UMFREVILLE PICKERING, M.A., F.R.S.,

Woburn Experimental Fruit Farm, Ridgmont, Beds.

THE effect of growing grass over the roots of fruit and other trees has been under investigation at the Woburn Experimental Fruit Farm, Ridgmont, since 1895, and the Thirteenth Report of that Farm, 1911, contained a general account of the results obtained up to that date.

There is no doubt that this deleterious action of grass varies greatly with the nature of the soil, though it is questionable whether in any case the effect would be nil: it is considerable even when the trees and grass are grown in pure sand, and fed with artificial fertilisers. It varies in extent with the nature of the trees, though none have yet been found which are not seriously affected by grass, under certain, and, generally, under most, circumstances. In the same way, the nature of the grass—eighteen varieties were examined—has only a minor influence on the results, the action of the weaker and shallower-rooted grasses being still very considerable.

At the Fruit Farm, which is situated on the Oxford clay, the effect of grass is nearly, and often actually, fatal to trees. Young trees grassed over at once after planting have their growth almost entirely arrested, and the grassing of trees which had been growing vigorously in tilled soil for four years, in one case, and for twelve years, in another, was found to produce the same result, the trees in the case of several varieties being actually killed. The effect of grass is noticeable even when a very small proportion of the roots of a tree are in grassed soil; but, on the other hand, recovery from the effect begins as soon as ever any of the tree-roots find themselves in tilled ground.

The stunting action of the grass is accompanied by other indications of starvation, the foliage and bark are of an unhealthy, light colour,

and there is a marked deficiency of green colouring matter in the fruit: but it is a case of starvation in a land of plenty, for, not only were all the experiments arranged so as to prevent the soil from being impoverished by the grass, but it has been found that the soil under the grass is actually richer than that in the tilled plots, and, if samples of two such soils are taken, and trees grown in them, those in that from the grassed plots exhibit about twice the vigour of those in the soil from the tilled plots.

The possibility of explaining the results by a difference in the water-contents of the grassed and tilled soil has been negatived by numerous experiments, both with trees grown in the open, and with others grown in pots, where the water-supply—and also the food-supply—could be strictly regulated. In some of these latter experiments the grass-roots were prevented from intermingling with the tree-roots by placing a piece of fine gauze between the two; yet in spite of this, and in spite, also, of all water and nutriment being supplied from below, so that the tree got all that it wanted before any reached the grass, the effect of the latter was nearly as great as in other cases.

Other possible explanations have been investigated, but have all been found to be insufficient: these included the questions of soil-temperature, alteration in the aëration of the soil, accumulation of carbon dioxide in it, its alkalinity or acidity, and any alteration in its physical nature. The apparent absence of other possibilities, combined with the general features of the action, led to the conclusion that this action must be due to some toxin produced by the grass: not necessarily, however, to any actual excretion from the roots, for a toxin might be produced either by the decay of the debris of the growing roots, or as a result of an alteration in the bacterial contents of the soil incident on the growth of the grass. It was possible, too, that the grass might become virtually toxic to the trees by taking up from the soil certain of its constituents, and thus modifying the character of nutriment available for the tree: but this possibility has been excluded by some experiments in which trees were grown in pots of earth, with trays containing growing grass resting on the surface: the trays were perforated, but the roots of the grass were prevented from penetrating through the holes by a layer of fine gauze; thus, if the grass still had an action on the tree it could not be due to anything being extracted by the grass from the soil in which the tree was growing, but must be due to what passed down from the grass to the tree in the water applied in watering the grass. That there was

such an action was undoubted: taking the three years' results over which these experiments extended, the average vigour of the trees with the trays of grass above them was only 73 per cent. of that of similar trees with similar trays of earth without grass; and the effect of the grass in the trays was not much less than that in cases where it was grown above the tree-roots with no trays; thus, where there was a sheet of gauze separating the grass-roots from the tree-roots the vigour of the trees was 71 per cent. of that of those without grass, and where there was neither trays nor gauze it was 60 per cent. It may further be added that the effect of the grass in these experiments was approximately the same whether the pots and trays both contained earth, or both sand; also that no certain difference was observed whether the trays contained grass germinating *in situ*, or grass which had been germinated some time before the trays were placed over the tree-roots.

In these experiments the leachings from the grass would reach the tree-roots in a very few minutes, but in two other sets of experiments the grass was grown in trays away from the trees, and the leachings were not applied to the latter till after they had been exposed to the air for an hour or two, an opportunity having thus been afforded for the oxidation of any toxin in them; here the results were the reverse of those in the former case, for the trees were benefited by the leachings to the extent of 30 to 40 per cent. in the two series.

In another instance where a toxic substance is certainly produced in soil, a precisely similar action occurs. The heating of soil produces an increase in the amount of soluble (especially organic) matter in it, and heated soil is found to be toxic towards plant-growth and seed-germination. On exposure to air and moisture, however, these toxic properties disappear, and, though the greater part of the soluble matter becomes insoluble, some of it is left in an oxidised form, and this renders the soil richer than soil which has not been heated at all. With soils heated to a high temperature, 125°—150°, and containing, therefore, a large amount of toxic matter, the effect of this persists throughout the whole period required for the growth of such plants as tobacco or tomatoes, but with soils heated to lower temperatures, the toxic effect is recognisable only in the early stages of growth, and the plants soon exhibit the beneficial effect of the oxidation product of the toxin. Grasses appear to be less sensitive to the toxic action, and, even in the case of highly heated soils, show the effect of it only in the early stages (this *Journal*, II. 411; III. 258, 277).

The results with trees were similar to those with other plants, and in spite of the greater time required for experiments with them, the deleterious effect of the toxin could be recognised by growing them with such a limited supply of air to the soil that the oxidation of the toxin was retarded (*Thirteenth Woburn Report*, 124). The increased growth eventually exhibited by plants in soils which have been only moderately heated cannot, however, be attributed solely to the effect of the decomposition product of the toxin, and another, and perhaps more important factor is the destruction by heat of the protozoa which feed on the bacteria, and the consequent increase after a time of the number of the latter.

The presence of the toxin in heated soil can be better investigated in germination experiments, which can be completed in a day or two, and these experiments give evidence of the presence of such toxin in soils heated to 60° only, whilst the general trend of the results indicates that traces of it may exist even in soils which have not been heated artificially at all; this is further supported by the observation that seeds germinate a little less readily in most soils than they do in pure silica. Germination experiments, however, have not afforded any evidence that soil under grass contains more toxin than tilled soil in its vicinity; on the contrary, seeds were found to germinate slightly more readily in the former. This might, of course, be explained by any toxin which was there having oxidised before the seeds had had time to germinate, so that the results cannot be accepted as affording evidence that such soils did not originally contain some toxin.

One suggestive point of similarity between grassed and heated soils is that both show a reluctance to become wetted when water is poured on them, this is very marked with some soils, especially after being highly heated, and it was distinctly observable with grassed soil in more than half the cases where such soil was compared with tilled soil taken in the same vicinity.

The experiments mentioned above in which trees were grown in pots with trays containing grass resting on the soil, have since been repeated in 1912 and 1913 with plants other than trees. The trays in this case were annular, and in 1913, both trays and pots were of earthenware. The internal diameter of the pots was 18 in., and their depth 13 in., whilst the central openings in the trays were $5\frac{1}{4}$ in. in diameter, and the depth of the trays 5 in. The soil in the pots weighed 31 kilos, and that in the trays 9 kilos, and the relative area of grass-grown soil in the tray to the total area of the pots was 1 : 2, or to that

TABLE II. *Series* 2 (*continued*).

	Plants in pots	Growth in tray	Dry weight of crop: Grams per plant	Dry weight of crop: Relative, blank = 100
	Effect of Clover. Ridgmont soil in pots and trays.			
	Tobacco, 2 plants	nil	33·2	100
29	,, ,,	Clover	1·0	3
30	,, ,,	,,	(6·8)	(21)
31	Tomatoes, 2 plants	nil	24·1	100*
32	,, ,,	Clover	7·1	29*
33	,, ,,	nil	38·9	100
34	,, ,,	Clover	24·6	63
35	Mustard, 4 plants	nil	40·8	100
36	,, ,,	,,	39·1	
37	,, ,,	Clover	31·4	77
38	,, ,,	,,	30·4	
	Effect of a plant on itself. Ridgmont soil in pots and trays.			
39	Festuca	nil	12·7 †	100
40	,,	,,	12·4 †	
41	,,	Festuca	2·5 †	24
42	,,	,,	3·5 †	
43	Clover	nil	3·2 †	100
44	,,	,,	2·6 †	
45	,,	Clover	0·6 †	16
46	,,	,,	0·5 †	
47	Mustard	nil	22·6 †	100
48	,,	,,	24·1 †	
49	,,	Mustard	7·0 †	40
50	,,	,,	11·3 †	
51	Dactylis	nil	6·0 †	100
52	,,	,,	6·0 †	
53	,,	Dactylis	1·1 †	31
54	,,	,,	2·5 †	

* Removed 10 days before 33 and 34 owing to damping having started. The mean of 32 and 34 is 46.

† Weights of total crops. In the case of mustard the weights per plant give the value of 52 instead of 40 for the experiments of mustard in the trays.

grown in the trays, these contained earth or sand, as the case might be, precisely similar to those where grass was grown. In 1912 nutrient was mixed with the soil in the pots, but only with that in those trays which contained grass, whereas in 1913 nutrient was mixed with the soil in all cases. The food material added was 2 per cent. calcium carbonate, 0·1 per cent. acid calcium phosphate, and 0·05 per cent. each of magnesium sulphate, potassium sulphate and sodium nitrate. All the water required to keep the pots up to a given weight (these being

weighed every other day) was put into the trays: when, in the blank experiment, these contained added food material, some of this would be washed down into the pot below, and this would tend to favour the plants without grass (1913 series), whereas, when only those trays in which grass was growing contained such extra food (1912 series), this washing out would favour the plants with grass. As will be seen below, the results were substantially the same in both series, and were not appreciably affected by these, or by any other differences in procedure.

The general results of the first series are given in Table I, and include nitrogen determinations kindly made by Dr Hutchinson at the Rothamsted laboratory. The results with the duplicates are satisfactorily concordant in most cases, except with the mustard, which was started somewhat too late in the season. The weights of the crop after drying at 100° C. (col. III) refer to the total crop, not to the weights per plant: these were not left till quite mature in all cases.

The portions of the plants above and below ground were examined separately, but did not add to the information obtained by taking the whole plants.

Series 2 are entered in Table II, the grass used being *Festuca pratensis*, instead of a mixture, as in Series 1. These experiments were all carried out under glass, where no differences in temperature would be caused by the surface growth. The number of plants grown in the pots in the experiments 1 to 38 was more limited than in the first series, and they were left till maturity. Nos. 39 to 54 were carried out at a later period, and the plants in them were more numerous, and did not attain maturity (with mustard there were 16 to 46 plants in the pots). The values given in brackets in this table will be considered later on.

The mean dry weights of the crops of grass, etc. in the trays were

3 & 4.	45 grams grass (2)		29 & 30.	103 grams clover (100)	
7 & 8.	66 ,, ,, (3)		32 & 34.	76 ,, ,, (5)	
11 & 12.	54 ,, ,, (4)		37 & 38.	87 ,, ,, (3)	
15 & 16.	72 ,, ,, (6)		41 & 42.	71 ,, Festuca (24)	
19 & 20.	48 ,, ,, (2)		45 & 46.	49 ,, clover (100)	
23 & 24.	49 ,, ,, (2)		49 & 50.	29 ,, mustard (3)	
27 & 28.	72 ,, ,, (6)		53 & 54.	19 ,, Dactylis (10)	

the numbers in brackets giving the proportion which the weights of the crops in the trays bear to those of the crops in the pots as unity.

Taking the results of both series together we get the following values for the crops when grass was grown in the trays, that where no grass was grown being taken at 100.

TABLE III. *Summary of results.*

Series	Crop	Contents of pot	Contents of tray	Relative weight of crop	
1	1. Tobacco	Ridgmont soil	Ridgmont soil	29	53
	2. Tomatoes	,,	,,	54	
	3. Barley	,,	,,	85	
	4. Mustard	,,	,,	42	
2	5. Tobacco	,,	,,	71	60
	6. ,,	Ashton soil	Ashton soil	52	
	7. ,,	Stackyard soil	Stackyard soil	63	
	8. ,,	Sand	Sand	55	
2	9. ,,	Ridgmont soil	Ridgmont soil	71	42
	10. ,,	,,	Ashton soil	65	
	11. ,,	,,	Stackyard soil	77	
	12. ,,	,,	Sand	35	
1	13. ,,	,,	,,	132	117
	14. Tomatoes	,,	,,	101	
	15. Barley	,,	,,	106	
	16. Mustard	,,	,,	128	
For the effect of clover we get					
2	17. Tobacco	Ridgmont soil	Ridgmont soil	3	42
	18. Tomatoes	,,	,,	46	
	19. Mustard	,,	,,	77	
For the effect of a crop on itself we get					
2	20. Festuca	Ridgmont soil	Ridgmont soil	24	28
	21. Clover	,,	,,	16	
	22. Mustard	,,	,,	40	
	23. Dactylis	,,	,,	31	

As to the general effect when a crop is grown in the trays, there can be no doubt; out of the 23 instances available there are only four where the effect is favourable. The first twelve instances show that the effect of grass in the trays is to reduce the crop in the pots to an average of about one half. The reduction, no doubt, varies with the nature of the crop, tobacco, according to the first four experiments, being most sensitive, and barley least so; but still greater variations are caused by other circumstances, so that the results of two similar experiments made at different times are not strictly comparable: thus, the results in the similar experiments 1 and 9, give 29 and 71, respectively, and those of 12 and 13, give 35 and 132. It is possible, however, to specify the cause of the difference between the last of these duplicates. They were experiments in which the grass was grown in sand: in 1913 the crop was a good one, and it was growing well before the plant-seeds were sown in the pots; consequently, it had a marked

effect on the plants (Nos. 8 and 12 in Table III): whereas in 1912, the grass crop was poor (less than half of that in the 1913 series, and two-thirds of that of the crop in trays of soil in 1912), and the plant-seeds were sown before the grass had become well established: the result was that its action on the plants was not deleterious (Nos. 13—16), and was, indeed, actually beneficial, a result which will receive an explanation later on.

Indeed, it hardly appears from the present experiments that the effect is materially altered by the nature of the medium, either in the pots or trays. In Nos. 5—8, cf. Table III, the same medium was used in both pots and trays, and the results show no systematic variation. The Ashton soil was from the Fruit and Cider Institute at Long Ashton, and was rich and heavy, the Stackyard soil was from that of the field of that name belonging to the Agricultural Society's Woburn Experimental Farm, and is very light and sandy. The same absence of definite variation exists in the results of Nos. 9—12, where various media were used in the trays, with Ridgmont soil in the pots.

Taking the results Nos. 5—12 with tobacco, which are strictly comparable *inter se*, and setting out the weights of the plants grown when there was no grass (*A*, in the table below) against the comparative weights of the crops when grass was present (*B*), it does not appear

No.	Actual crop *A*	Relative crop under grass *B*	Weight of grass in trays *C*
11	33	77	35
9	33	71	42
10	33	65	47
7	25	63	48
8	23	55	60
6	40	52	66
12	39	35	55

that the grass-effect bears any direct relation to the vigour of the plant in the medium used; for instance, the effect (*B*) is nearly the same in Nos. 8 and 6, though the growth of the plants without grass (*A*) was nearly twice as great in the one case as in the other. When, however, the crops in the pots under grass (*B*) are compared with the crops of grass in the trays above them (*C*), it is seen that these two, with the exception of the last entry, vary in opposite directions. The different behaviour of grass in sand in the two series quoted above, may be cited as a further illustration of the dependence of the

effect on the magnitude of the crop of grass, and yet another illustration is found in former experiments, where the effect of 18 different grasses on apple trees was examined, and the magnitude of this effect was shown to vary with the vigour of the grasses (*Thirteenth Woburn Report*, p. 45).

The Long Ashton soil was used in these experiments because it had been found that the action of grass on apple trees growing in it, though recognisable on careful measurement, was comparatively small. The soil, however, as is here seen, behaves in the same way as the other soils under similar circumstances, and the different behaviour of apple trees at the place itself is probably attributable to the great depth of the soil existing there (see below, p. 149).

The crops under grass, being smaller than those without grass, will have removed less nitrogen from the soil, for, as seen by the values in Cols. IV and VII of Table I, the nitrogen removed is closely proportional to the weight of the crop; consequently there must be most nitrogen left in the soil of the pots where the trays contained grass. The analyses bear this out, although they are not very concordant: taking the four cases dealt with in Table I, the soil of the grass-grown pots contained altogether 7·39 grams of nitrogen, against 5·56 grams where there was no grass. Thus, the soil is actually richer where the plant growth is less, and this reduced growth can only be due to some toxic action preventing the plants from utilising the nourishment present. This is further confirmed by the results where the trays contained sand: in Series 1 the grass had no deleterious effect when grown in sand, and, in harmony with this, we find that the soil in the pots contained the same amount of nitrogen: 6·15 grams where no grass was grown, 6·17 grams where it was grown.

The effect of clover (Nos. 17—19, Table III) is, on the whole greater than that of grass (Nos. 1—4), but that is chiefly due to the all but lethal effect which it had on tobacco (No. 17); on mustard it had less effect than grass. The clover crop in the trays was greater, however, with the tobacco (103 grams) than in the other two cases, where it was 77 and 87 grams, respectively.

The effect of a crop in the trays on a crop of the same nature in the pots is shown by Nos. 20—23, Table III, to be considerably greater than is its effect on other plants: the growth is reduced from 100 to an average of 28; and here, again, clover has the most marked effect, causing a reduction to 16.

The grass in the trays produced no appreciable effect on the time of

Tobacco.

Without grass in trays. With grass in trays.

Tobacco

Without clover in trays. With clover in trays.

Festuca pratensis.

Festuca in trays. No festuca in trays.

germination of the seeds in the pot, but the effect on the growth of the plants was manifest from the earliest stages up to the conclusion of the experiments. This was shown by the weights per plant of such plants as were thinned out from time to time. The results with tobacco were most complete, and may be thus summarised, the numbers in brackets giving the weights per plant after drying. No. 8 (sand in trays) is omitted, as the thinnings were not taken at the same time as the others, owing to the backwardness of the plants.

No. Table III	1st thinning	2nd thinning	3rd thinning	Final plants
5	62 (·009)	27 (·087)	33 (·49)	71 (23·4)
6	47 (·006)	22 (·054)	29 (·29)	52 (20·9)
7	38 (·004)	71 (·052)	21 (·21)	63 (15·4)
10	35 (·007)	98 (·093)	32 (·30)	65 (21·9)
11	19 (·006)	55 (·099)	12 (·71)	77 (25·8)
12	38 (·004)	81 (·043)	47 (·53)	35 (13·5)
Mean ...	40	59	29	61

One more important fact remains to be noticed, and this seems to give an explanation of the whole phenomena. In all the experiments discussed so far, the crops from the pots with and without grass were removed at the same time, and in most cases those without grass had fully matured and were fruiting when removed: but those with grass, owing to the toxic action of this latter, had not attained maturity. In the first 30 experiments in Table II, one only of the duplicates with grass was in each case removed at the same time as those without grass, whilst the other was left to mature, which it did about a month later. The values given by these latter are enclosed in brackets in Table II, and, as will be seen below, they show that the plants had (except in one case) not only made up for lost lee-way, but had actually outstripped those without grass.

No. Table II		Comparative growth of	
		Immature plants	Mature plants
3 & 4.	Tobacco with grass	71	120
7 & 8.	,, ,, ,, ...	52	107
11 & 12.	,, ,, ,, ...	63	127
15 & 16.	,, ,, ,, ...	55	139
19 & 20.	,, ,, ,, ...	65	135
23 & 24.	,, ,, ,, ...	77	145
27 & 28.	,, ,, ,, ...	35	113
29 & 30.	,, ,, clover	3	21

The conclusion from this is obvious: the effect of the toxin formed by the grass is eventually overpowered by the beneficial effect of some other substance formed, which other substance is, doubtless, as in the case of heated soils, merely the oxidation product of the previously formed toxin itself. This is in harmony with the observations recorded above that the leachings of growing grass, if oxidised, are beneficial, not toxic, and that the soil removed from grassed ground is more favourable to the growth of trees than that from similar tilled ground.

Recovery from the toxic effect may not always occur, for this effect may have been so great that the plant is permanently injured. This was apparently the case with the tobacco plants under clover. With hard-wooded plants, also, recovery is improbable, for a severe check to growth during their early years leads to permanent stunting, from which, as is well known, they rarely recover. This is why no instances of recovery from the grass effect have been noticed under ordinary circumstances with fruit trees at the farm. But it explains the recovery which is being noticed there in one exceptional case where the grassing occurred gradually throughout several years, and where the check to growth was much less than in the other cases where the ground was grassed at once (*Thirteenth Woburn Report*, p. 25).

It is even possible that partial grassing might have a beneficial effect, when the grass is at such a distance from the tree-roots that the toxin becomes oxidised before reaching these roots. One experiment at Woburn shows that this is the case. Standard apple trees were planted in grassed land, with the grass removed to different distances from the stems, and, as will be seen from entries below, there was actually a beneficial effect where the grass was 3 feet from the stems (and to a much smaller extent where it was 6 feet), but this effect disappeared after two years as the roots extended, and found themselves under the immediate effect of the grass.

Growth of apples with the grass removed to different distances from the stems.

Year	No grass	Grass 6 feet away	Grass 3 feet away	Grassed up to stems
1910	100	104	132	77
1911	100	108	168	30
1912	100	82	78	6
1913	100	61	61	5

The beneficial action of the oxidised toxin is also the explanation of the increased growth observed in 1912 when a poor crop of grass was grown in trays containing sand (p. 144), whilst the toxic effect was apparent when there was a better crop of grass (as in 1913), which would render aëration more difficult. This would be the case, too, with the experiments wherein trees were grown under grass in sand (p. 138); for long before the end of the three years throughout which the experiments lasted, the sand would have become too clogged to admit of easy aëration.

The present explanation of the grass effect is quite in harmony with the great variations exhibited under different conditions. The toxic action will be increased where the grasses are of the stronger growing class, as has been found to be the case (p. 146), and will be less effective on the stronger growing varieties of trees, which has also been found to be the case at Woburn. It must necessarily vary with the character of the soil: if this is rich, or of great depth, or if it favours the oxidation of the toxin, this latter will be less injurious: at the Woburn farm the soil is shallow, not very rich, and very difficult to aërate, consequently the toxic action is great: at Long Ashton the reverse conditions obtain, and the toxic action is small. In the only case where we have noticed no toxic action (at Harpenden, *Thirteenth Report*, p. 4) the soil is very rich, being old garden soil. Even at Ridgmont, manure lessens the toxic action, though it does not by any means do away with it (*loc. cit.* p. 71). In the pot experiments with tobacco, it may be objected, no connection between the extent of the toxic action and the richness of the soil was noticed, but in that case ample food material for the growth of the plants was added in all instances.

In short, all the observations made at Woburn during the last eighteen years are in harmony with the explanation of the grass-effect now given.

In the accompanying plate are reproduced photographs of three of the sets in the 1913 series, taken just before the plants were turned out. One of the duplicates of tobacco with grass in the trays was left, and, as stated, the plants eventually outstripped those without grass, but unfortunately no photograph of the pot was taken at this later date. In the centre figure the tobacco is so dwarfed that it does not appear above the rim of the pot.

The experiments on the effect of a crop on itself have not yet been extended so as to show whether recovery will eventually take place in such cases also. No doubt it often will, but seeing that the toxic effect

is greater than where the plants are different, it will be slower, and may not always occur. It is a well-known fact that with agricultural crops, especially when arranged in well-defined plots, the plants in the centre of a plot are at first less vigorous than those in the outside rows, and this may be attributed to the toxic action of the neighbouring plants being only half as great in the outside rows as in the centre of the plot: but this is noticeable only in the early stages of growth; when the crops are mature such differences seem to disappear (though exact observations on this point are wanting), the toxic effect possibly having given place to the beneficial effect of the oxidised toxin.

The bacterial features of the soils in our pot experiments are being investigated by Dr Hutchinson. The results will, doubtless, be of considerable interest, and relations will probably be found to exist between the bacteria and the behaviour of the plants with and without grass. But it will be difficult to determine whether bacterial alterations are the prime cause of the altered plant-growth, or merely incidental accompaniments of the soil conditions. So far as the results published at present indicate, the alterations in plant-growth cannot in this case be attributed primarily to bacterial causes: in the pots with grass there are more bacteria than in those without (*loc. cit.* p. 123), this would indicate a greater richness of soil with grass (which does exist), and a more vigorous plant-growth (which does not exist); moreover the toxic effect does not follow the mere number of bacteria present, for, where sand is used, the number present is less in the grassed pots (where the plant-growth is stunted), than it is in the ungrassed pots containing earth (where the growth is vigorous).

In the case of the heated soils, the toxin is formed by the action of the heat alone, and the subsequent oxidation of the toxin can occur without the intervention of bacteria (this *Journal*, III. 269): there is no reason to suppose that the changes in the organic debris of a growing crop may not equally occur without the agency of bacteria, though in all probability they may be materially aided by these organisms.

SUMMARY.

Every growing crop results in the formation of a substance which is toxic to the growth of other plants, and still more so to itself.

By oxidation this toxin loses its toxic properties and enhances the fertility of the soil. The plants previously poisoned eventually outstrip those which had not been subjected to the poisoning, except in cases where the toxic effect has been sufficient to produce a permanent stunting.

The toxic effect must necessarily vary considerably with the different conditions obtaining, both as to the nature of the soil, the plant affected, and the vigour of growth of the plant producing the toxin.

There is no reason for assuming the excretion of any toxic matter from a plant, the debris from the growing roots is probably sufficient to account for the formation of the toxin.

The heating of a soil produces toxic matter from the organic substances present in it, and in much greater quantities than that produced by the growth of a crop. In both cases the toxin, after oxidation, increases the fertility of the soil.

METHODS OF ESTIMATING CARBOHYDRATES. II. THE ESTIMATION OF STARCH IN PLANT MATERIAL.

THE USE OF TAKA-DIASTASE.

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IN 1884 C. O'Sullivan (*Trans. Chem. Soc.*, 1884, **45**, 1) described a method of estimating starch in cereals and other starch-containing substances which was based on the conversion of starch by means of diastase into a mixture of "dextrin" and maltose. This method has been improved at various times by H. T. Brown and his fellow workers, especially by Brown and Millar (*Trans. Guinness Research Lab.*, 1903, 79) in its application to malt and barley. Having encountered difficulties in applying this method to the estimation of starch in leaf material, we have been led to make a careful study of the various processes available for this purpose and to devise a method to obviate the errors to which the ordinary diastase method is subject in such cases.

For the estimation of starch in plant materials, such as foliage leaves, seeds, grain, etc., the modified Sachsse method which is official in the United States of America (*Bureau of Chemistry*, Bulletin 107; *Allen's Commercial Organic Analysis*, 4th Ed., Vol. I. p. 420) and is based on the hydrolysis of the starch present with boiling dilute hydrochloric acid, is quite valueless, not only because such tissues invariably contain pentosans and other substances which yield reducing sugars that count as dextrose, but because of the actual destruction of dextrose which occurs during the prolonged treatment with acid[1].

[1] We have found in a series of analyses made by this method of purified potato starch dried *in vacuo* at 120° C. (see p. 167), results varying from 93·8 to 94·3 °/₀ of starch, whereas by the ordinary diastase method an average result of 100·1 °/₀ (see p. 165), and with taka-diastase an average result of 99·6 °/₀ was obtained with the same sample. That actual destruction of dextrose occurs on prolonged heating with dilute acid we have recently shown (*Journ. of Agric. Sci.*, 1913, **5**, p. 437). This destruction of dextrose is a source of error in all the methods which make use of hydrochloric acid to effect hydrolysis, such as that of Märcker and Morgen, even when the primary conversion of the starch has been carried out with diastase.

Although ordinary diastase gives with purified starch results by O'Sullivan's method which are approximately correct (see p. 165), values 15 to 20 °/₀ lower than the actual starch content may be obtained when it is applied to leaf material or plant tissues in general, owing to the loss of dextrin. In the majority of cases, plant material, which has been previously deprived of sugar by prolonged extraction with alcohol, still contains tannins, amino-acids, proteins, etc.; during the hydrolysis by diastase these pass into solution and exercise a very marked effect on the reducing power and optical activity of the solution. These substances have therefore to be removed by the addition of basic lead acetate, which we have found almost invariably produces a heavy precipitate in the filtered solution obtained from the diastase conversion. Although basic lead acetate *does not of itself precipitate dextrin,* when dextrin is present in solutions in which a precipitate is produced, as in the purification of the solutions obtained from the diastase conversions, it is *carried down with this precipitate and is thus lost to the analysis*[1].

Taka-Diastase as an Agent in Estimating Starch.

To estimate starch in foliage leaves and in similar cases in which it is necessary to purify the solution after hydrolysis has been effected, it appeared probable that the so-called "taka-diastase" would be more suitable than ordinary diastase, as it is said to give rise only to maltose and dextrose (compare Croft-Hill, *Proc. Chem. Soc.*, 1901, **240**, 184) free from dextrin[2]. If this were the case, it would be possible to add basic lead acetate or other clarifying agents without losing sugars. We therefore carried out a series of experiments with taka-diastase at two different temperatures, namely 38° and 55° during different lengths of time. In all cases, about 2·5 grms. of potato starch were

[1] When basic lead acetate is added to the solution obtained by the diastase conversion of *purified starch* not the slightest precipitate is produced with the dextrin existing in solution; but the results given on pp. 165–167 show that if sodium carbonate is subsequently added, or hydrogen sulphide is passed so as to precipitate the lead, a greater or smaller proportion of the dextrin is removed by co-precipitation.

[2] In 1898 Stone and Wright (*J. Amer. Chem. Soc.* **20**, 639–647) attempted to estimate starch by means of taka-diastase; but as they assumed maltose to be the only sugar formed and measured the products of the action solely by the reducing power without reference to their rotation, it is not surprising that they concluded that under their conditions "taka-diastase is not adapted for use in the quantitative estimation of starch." It will be seen that we have come to an exactly opposite opinion.

dried *in vacuo* over phosphorus pentoxide at 120° in a small wide-necked, round bottomed flask, fitted with a ground-in stopper carrying a tube connected with the vacuum through a small Wurtz flask containing the pentoxide; the heating was effected by means of a Meyer vapour bath containing a mixture of toluene and xylene which gave the required temperature. The starch was dried until the weight was constant; 2·5 grms. of the original starch gave approximately 2·0 grms. of the vacuum-dried product. This was gelatinised with 200 c.c. of water at 100°, the solution cooled to the proper temperature and mixed with 0·100 grm. taka-diastase[1]; the volume was kept at 200 c.c. by the addition of water as required and the temperature was maintained constant at 38 or 55° to within 0·1° by means of a thermostat. When the action was prolonged over more than a few hours, toluene was added in sufficient quantity to prevent the growth of micro-organisms; it was thoroughly mixed with the solution by stirring and from time to time fresh quantities were added so as to maintain the material in a sterile state. Chloroform of course cannot be used as it destroys the maltase present in taka-diastase (compare Fischer, *Ber.*, 1895, **28**, 1429, and Morris, *J. Fed. Inst. Brewing*, 1896, 350). Ultimately, the solution was boiled to destroy the diastase or a trace of sodium hydroxide was added; it was then diluted to 500 c.c. and the reducing power estimated in 25 c.c.

The observations of rotatory power were made in a 200 (or 400) mm. tube at 20·00°; the specific rotatory power of anhydrous maltose[2] is $[\alpha]_D^{20°} = 137·6°$, that of dextrose in dilute solution $[\alpha]_D^{20°}$ being taken as 52·7°.

From Table I it is seen that when the time of action of the taka-diastase at 38° under the conditions given above exceeds six hours, the whole of the starch is converted into a mixture of maltose and dextrose, the proportion of dextrose steadily increasing as time proceeds, so that the ratio of dextrose to maltose present, which after six hours is approximately 0·1, increases to about 6·2 after 72 hours. At this point, under the conditions existing in these experiments (namely starting with 0·1 grm. only of taka-diastase), no further conversion of maltose into dextrose occurs.

The curves (Fig. 1, p. 156) show the results graphically.

[1] We have used the commercial product manufactured by Messrs Parke Davis & Co.

[2] Applying Meissl's temperature correction to the value $[\alpha]_D^{15·5°} = 137·93°$ obtained by Brown, Morris and Millar (*Trans. Chem. Soc.*, 1897, **71**, p. 112) as the specific rotatory power of pure maltose.

TABLE I. *Action of* 0·1 *grm. Taka-Diastase on Potato Starch at* 38°.

Time in hours	Weight of starch dried in vacuo at 120°	CuO from 25 c.c. of 500 c.c.	α_D in 200 mm. tube at 20·00°	Dextrose in 500 c.c.	Maltose in 500 c.c.	Total starch	% starch found	Dextrose / Maltose	Remarks
3	2·0061	0·1321	1·192°	—	—	—	—	—	Dextrin still present. No alumina cream added
	1·9978	0·1380	1·273	—	—	—	—	—	
6	2·0099	0·1581	1·103	0·2204	1·892	1·9914	99·55	0·116	No alumina cream
	1·9914	0·1535	1·097	0·1640	1·932	1·9776	99·34	0·085	,, ,, ,,
12	1·9752	0·1780	0·991	0·5328	1·5930	1·9884	100·7	0·334	,, ,, ,,
	1·9940	0·1800	0·973	0·5748	1·5430	1·9792	99·37	0·372	5 c.c. alumina cream
24	2·0148	0·2189	0·808	1·1596	1·0214	2·0112	99·80	1·135	,, ,, ,,
	1·9876	0·2205	0·764	1·2302	0·9148	1·9739	99·33	1·345	,, ,, ,,
	2·0145	0·2218	0·783	1·2204	0·9534	2·0018	99·37	1·280	No alumina cream
	2·0046	0·2212	0·777	1·2218	0·9414	1·9916	99·39	1·298	5 c.c. alumina cream
	2·0024	0·2167	0·794	1·1546	0·9982	1·9846	99·11	1·157	5 c.c. basic lead added and this pptd. by solid Na_2CO_3
48	2·0000	0·2561	0·594	1·8106	0·3852	1·9942	99·71	4·700	No alumina cream
	2·0000	0·2544	0·616	1·7674	0·4416	2·0086	100·40	4·002	5 c.c. alumina cream
72	2·0016	0·2588	0·556	1·886	0·2874	1·9703	98·45	6·563	,, ,, ,,
	1·9961	0·2562	0·562	1·852	0·3113	1·9620	98·28	5·950	,, ,, ,,
	2·0084	0·2571	0·569	1·8536	0·3231	1·9741	98·33	5·737	,, ,, ,,
	2·0046	0·2568	0·566	1·8538	0·3178	1·9696	98·27	5·833	,, ,, ,,
96	2·0000	0·2592	0·562	1·8838	0·2992	1·9790	98·95	6·296	,, ,, ,,
	2·0048	0·2590	0·572	1·8694	0·3226	1·9879	99·15	5·794	Nothing added
	2·0054	0·2569	0·562	1·8600	0·3082	1·9660	98·05	6·034	5 c.c. alumina cream

TABLE II. *Action of* 0·1 *grm. Taka-Diastase on Potato Starch at* 55°.

Time in hours	Weight of vacuum dried starch	CuO from 25 c.c. of 500 c.c.	α_D in 200 mm. tube at 20·00°	Dextrose in 500 c.c.	Maltose in 500 c.c.	Total starch	% starch found	Dextrose / Maltose	Remarks
12	2·0030	0·1578	1·074°	0·2366	1·8564	1·9716	**98·46**	0·127	Nothing added
24	1·9617	0·1597	1·059	0·2738	1·8146	1·9655	**100·2**	0·151	,, ,,
48	2·0171	0·1718	1·026	0·4506	1·6576	1·9758	**98·0**	0·272	5 c.c. alumina cream

The dextrose curve represents the dextrose formed from 100 grms. of starch, *calculated as starch* (by multiplying the dextrose figure at each time by 0·9); the maltose curve similarly represents the maltose as starch (dividing the maltose figure by 1·055). This system of

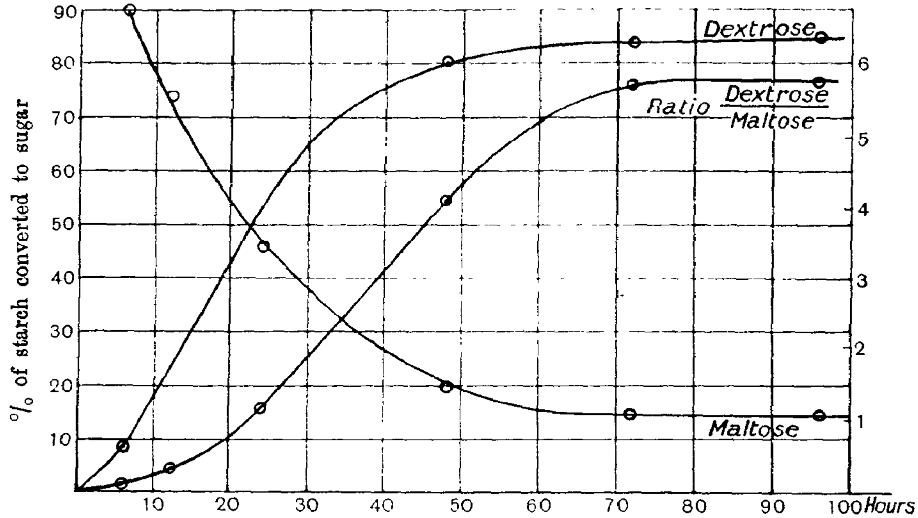


Fig. 1.

plotting the results has the advantage of showing for every moment the *proportion of the original starch which is present either as maltose or dextrose,* the sum of the maltose and dextrose values at each point being approximately 100, so that the two curves are complementary. The actual values plotted are as follows, being the mean values calculated for each time from the series in Table I.

TABLE III.

Time in hours	Starch present as dextrose	Starch present as maltose	Maltose starch / Dextrose starch
6	8·8 %	90·8 %	0·1
12	25·0	74·5	0·33
24	54·0	45·8	1·18
48	80·5	19·6	4·10
72	83·9	14·8	5·66
96	84·2	14·7	5·72

From the shape of the curves, coupled with the results found after three hours, it is probable that the first action of the taka-diastase is to break down the starch to dextrin and maltose, just as in the case of

ordinary diastase; the maltase comes into action comparatively slowly, so that after six hours only $\frac{1}{10}$th of the original starch is present as dextrose. Subsequently, however, the rate at which dextrose is formed increases, following very nearly a straight line curve between six hours and 28 hours, when about 60 °/₀ of the starch is present as dextrose; the rate of formation of dextrose then rapidly slows down until a nearly constant value is reached in the neighbourhood of 84 °/₀.

This arrest-point (possibly an equilibrium position) is very similar to the arrest-point which is reached in the case of ordinary diastase, at 55°, when about 80 °/₀ of the starch has been converted into maltose and 20 °/₀ remains in the form of dextrin. It might be thought from the coincidence of the numbers, that with taka-diastase the same proportion of a resistant dextrin is formed which then remains unchanged whilst the maltose is converted into dextrose, but such an assumption does not accord with the values actually found for the reducing power and polarisation at different times given in Table I. Dextrin does not appear to be present after about four hours.

That the product of conversion consists solely or almost solely of the sugars dextrose and maltose, as seems highly probable from the results of Table I, is borne out by the following actual estimations of maltose by the use of maltase-free yeasts (Davis and Daish, *J. Agric. Sci*, 1913, **5**, 462).

2·1671 *grms. vacuum dried starch digested* 36 *hours at* 38° *with* 0·1 *grm. taka-diastase; made up to* 500 *c.c.*

CuO from 50 c.c.	α_D in 400 mm. tube at 20°	CuO from 50 c.c. after fermentation with		Maltose calculated	
		S. Exiguus	*S. Marxianus*	from direct CuO and rotation	from CuO after fermentation
0·4496	1·317°	0·1243	0·1210	0·0833	0·0893

From Table II it is seen that *the action of the enzyme maltase which converts maltose into dextrose is very much restricted at* 55°; on comparing the numbers showing the ratio of dextrose to maltose with those for the same periods at 38° (Table 1), it would appear that the enzyme is gradually destroyed at the higher temperature. The destruction, however, does not take place very rapidly, and although the action is decidedly limited after 24 hours, the increase in the ratio of dextrose to

maltose from 0·15 to 0·27, which occurs after 48 hours, shows that maltase still persists and exercises some activity.

As regards the applicability of taka-diastase to the analytical estimation of starch, the values given for the percentage of pure starch found in the conversions from six to 48 hours at 38° agree fairly closely among themselves, although they cover a wide variation in the ratio of dextrose to maltose (namely from 0·1 to 4·7); it thus seems highly probable that maltose and dextrose are the sole products of the conversion. For analytical purposes at least the error likely to be incurred by this assumption is small. It is noteworthy that the average value for the starch present in the purified potato starch found on using taka-diastase in the conversions of six to 48 hours is 99·65, whereas with ordinary diastase a value generally 0·5 per cent. higher was obtained (see p. 165). It is probable that the numbers obtained with taka-diastase more nearly represent the true starch values, as the calculations are based on the constants for two pure sugars only; they do not involve any assumption with regard to specific rotatory power for the "dextrin" existing in solution, which is generally taken at 202°, but some doubt may still be entertained as to the exactness of this value. It is probable too that the purified potato starch contains a small proportion of foreign material; hence the low value 99·6 °/₀.

When the conversions with taka-diastase are prolonged beyond 48 hours, somewhat lower starch values are generally obtained, as is seen in Table I; it is possible that some slight destruction of the sugars may occur during these prolonged conversions, but the lower values may also be due to the fact that a larger proportional error is incurred in the reading of the rotation. In Table I, the actual readings in a tube of 200 mm. for the longer conversions range only from about 0·5 to 0·6°; an error of 0·005° in the reading would therefore represent an error of 1 °/₀. That there is the actual closeness of concordance between the results for starch given in Table I with such small angles of rotation is due to the fact that with the instrument we have in use it is possible to read the rotation with a probable error not exceeding 0·005°. The use of taka-diastase in starch estimations has the advantage that it gives rise to two *sugars*, maltose and dextrose, the rotatory powers of which have been carefully determined; the temperature coefficients for these are exceedingly small so that no very special precautions to ensure exact constancy of temperature are necessary in ordinary work.

In actual analytical practice it is an easy matter to arrange the

quantities so that considerably higher rotations are observed and the proportional error in this direction minimised; examples of this are given on p. 162. That the addition of precipitating agents such as alumina cream and basic lead acetate, and the formation of heavy precipitates, such as are obtained with lead and sodium carbonate, do not in the least influence the results is shown by the examples in Table I, and by numerous other analyses which we need not quote.

Application of Taka-Diastase to Plant Material.

In estimating starch and the other carbohydrates in plant material, it is most essential that *the enzymes should be destroyed immediately after the sample* is taken, so as to prevent subsequent change in the relative and absolute proportions of the carbohydrates present. Unless this precaution be observed results are obtained which do not in the least represent the amount of actual carbohydrates present in the original tissue. Thus, for example, if the leaf tissue be dried in a drying oven before destroying the enzymes, during the slow loss of water from the leaf material and before the enzymes are paralysed considerable change may occur.

By dropping the leaf or other material directly into a large volume of boiling 95 °/$_0$ alcohol to which 1 °/$_0$ of its volume of 0·880 ammonia has been added, all plant enzymes seem to be immediately destroyed; no change in the proportions of the sugars present in the extract takes place subsequently even if the solutions are kept several weeks, when proper precautions are taken to exclude air and a little toluene is added to prevent the growth of micro-organisms.

Before the starch can be estimated in the leaf or other tissue, it is necessary to remove the sugars completely; for this purpose the leaf tissue, after killing the enzymes, is extracted for 18 to 24 hours with boiling alcohol in a special large form of Soxhlet extractor which will be described in a subsequent paper. Experiments have shown that the extraction of the sugars from leaves is complete as soon as the green colour of the chlorophyll has been removed. After the extraction is finished, the leaf material is pressed free from alcohol in a powerful screw hydraulic press (for example, of the Buchner type), and is thus obtained free from traces of the sugars, in a form in which it can be rapidly dried. The pressed cake is broken up into shreds and the material so obtained dried in a steam oven for 18 hours. It is then rapidly ground in a small mill and bottled for analysis

Our starch analyses in plant material are always referred to *vacuum dried weights*. For this purpose about 10 grams of the oven dried extracted material is dried to constant weight at 100 or 110° *in vacuo* over phosphorus pentoxide in the apparatus referred to on p. 154. It is generally necessary to heat the material for 24 hours or even longer.

To estimate starch, the dry material (free from sugars and, if necessary, previously extracted with water to remove gums, amylans, etc., see p. 162) is gelatinised with 200 c.c. of water in a beaker flask heated for ½ hour in a water bath at 100°. The solution is cooled to 38°, 0·1 grm. taka-diastase added, together with 2 c.c. of toluene and the mixture left 24 hours in order that the conversion may take place; it is then heated in a boiling water bath to destroy the diastase and the clear solution above the residual leaf material is filtered through a fluted filter paper into a 500 c.c. measuring flask; the leaf residue is thoroughly washed several times by decantation, the washings being passed through the filter paper until the volume of liquid in the flask amounts to about 475 c.c. The necessary quantity of basic lead acetate is then added to precipitate the tannins, etc. present in the solution; the amount required varies considerably with different leaves, generally ranging from 5 c.c. to 25 c.c. A large excess of lead should be avoided and tests should be made after each small addition of lead acetate in order to ascertain when the precipitation is complete. When this is the case the solution is made up to 500 c.c. at 15°, and filtered; 100 c.c. of the filtrate is placed in a 110 c.c. measuring flask, the slight excess of lead precipitated by adding solid sodium carbonate and the volume adjusted to 110 c.c. at 15°. 50 c.c. of the filtrate from the lead carbonate is used for the reduction and another portion polarised in a 400 mm. tube. The following example shows the method of calculation:

Weight of extracted leaf material (*Tropaeolum majus*) after drying in steam oven	= 10·4122 grms.
Weight of leaf material dried in vacuo at 100°	= 9·4059
CuO from 50 c.c. of the final 110 c.c.	= 0·4492 grm.
Polarisation of this solution in 400 mm. tube at 20·00°	= 1·995°

If x = grms. dextrose in 50 c.c. of this solution
y = grms. maltose ,, ,, ,, ,,

we have, using the values of CuO corresponding to 1 grm. of dextrose and maltose for the weight 0·4492 CuO in the tables of Brown, Morris and Millar:

$2{\cdot}369x + 1{\cdot}362y = 0{\cdot}4492$ ... ... (1)

For the 400 mm. tube, employing the values $[\alpha]_D^{20^\circ} = 137{\cdot}6$ and $[\alpha]_D^{20^\circ} = 52{\cdot}7$ for maltose and dextrose we have also

$4{\cdot}216x + 11{\cdot}008y = 1{\cdot}995^\circ$ ... ... (2)

Solving equations 1 and 2 for x and y

$x = 0{\cdot}1095$ grm. dextrose in 50 c.c.
$y = 0{\cdot}1394$ grm. maltose in 50 c.c.

Total dextrose in 500 c.c. original solution $= 0{\cdot}1095 \times \frac{110}{50} \times \frac{500}{100} = 1{\cdot}2045$ grm.

,, maltose in ... ... $= 0{\cdot}1394 \times \frac{110}{50} \times \frac{500}{100} = 1{\cdot}5334$ grm.

Starch corresponding to dextrose $= 0{\cdot}90 \times 1{\cdot}2045 = 1{\cdot}0840$ grm.

,, ,, ,, maltose $= 1{\cdot}5334 \div 1{\cdot}055 = 1{\cdot}4535$,,

Total starch $= 2{\cdot}5375$ grms.

∴ °/o of starch in vacuum dried extracted leaf material

$$2{\cdot}5375 \times \frac{100}{9{\cdot}4059} = 26{\cdot}97\ {}^\circ\!/_\circ.$$

Precautions necessary in taking Leaf Samples for Analysis.

If the dried, ground leaf material is bottled before analysis, it is absolutely necessary when each sample is taken for the analysis, *to turn out the whole of the material on to a sheet of paper and thoroughly mix it before sampling.* If this precaution be not observed and successive samples are taken directly from the bottle, it is found that the proportion of starch present in the material increases towards the bottom of the bottle. This is no doubt due to the fact that the heavier starch grains, set free from the tissue by grinding, sink to the bottom of the bottle, whilst the lighter fibrous material of the leaf rises to the top. This is well shown by the following successive analyses made with potato-leaves (previously freed from sugars by extraction):

1	sample from top of bottle, starch	= 7·54 °/o	on vacuum dried matter	
2	,, ,, middle of bottle	= 9·19 °/o	,,	,,
3	,, ,, ,, ,,	= 9·23 °/o	,,	,,
4	,, ,, bottom ,,	= 12·29 °/o	,,	,,

Several similar results were obtained with the leaves of turnips, *Tropaeolum*, etc. which we need not quote, before we became aware of the necessity of special care in the sampling of the finely ground leaf material. When, however, the sampling is carried out in the way described, the agreement between different individual determinations is as satisfactory as could be expected in this class of work. We append a few examples.

TABLE IV. *Turnip leaves. Sample taken* 4.45 *p.m., July 9th*, 1913, *bright but cool day. Starch in vacuum dried leaf after extraction of sugars.*

Weight of vacuum dried material	CuO from 50 c.c. of 110 c.c.	a_D in 400 mm. tube at 20·00°	Total maltose	Total dextrose	Total starch	% starch in vacuum dried leaf	Dextrose / Maltose	Remarks
10·0705	0·3033	1·661°	1·4487	0·5412	1·8601	**18·47**	0·374	24 hrs. conversion. Required 12·5 c.c. basic lead acetate
9·8300	0·3456	1·439	1·0736	0·9454	1·8690	**19·01**	0·880	27 hrs. conversion. 12·5 c.c. basic lead acetate
9·2455	0·3000	1·448	1·1880	0·6689	1·7290	**18·71**	0·563	
					Average	**18·73**		

TABLE V. *Nasturtium leaves* (Tropæolum majus). *Dry leaf after extraction of sugars. Sample taken July* 11, 1913, 5.15 *p.m. after fairly sunny day.*

Weight of vacuum dried material	CuO from 50 c.c. of 110 c.c.	a_D in 400 mm. tube at 20·00°	Total maltose	Total dextrose	Total starch	% starch in vacuum dried leaf	Dextrose / Maltose	Remarks
9·4059	0·4492	1·995°	1·5334	1·2045	2·538	**26·97**	0·786	24 hrs. conversion. 15 c.c. lead acetate required
9·0404	0·4368	1·823	1·3398	1·2518	2·397	**26·53**	0·934	
					Average	**26·75**		

Mangold Leaves. Very numerous analyses made with mangold leaves sampled at various periods of the night and day have shown that during the period when cane sugar is being actively stored by the roots, starch is entirely absent from the leaf.

The Necessity of removing Substances soluble in Water other than Sugars which are optically active.

One of the principal difficulties in estimating starch in plant material is due to the presence of gummy substances, tannins, proteins, etc. which pass into solution during the hydrolysis and exercise an effect on the rotatory and reducing power of the solution. These substances are very largely removed by the use of basic lead acetate, but sufficient impurity remains, even after this treatment, to falsify the

analyses in some cases, as shown by the following results obtained with mangold leaves free from starch[1]:

9·6205 grms. vacuum dried leaf was treated with 0·1 grm. taka-diastase as in an ordinary starch estimation; after digestion for 36 hours at 38°, the solution was filtered and washed to 470 c.c., basic lead acetate being then added until no further precipitate was obtained. The solution was made up to 500 c.c., filtered and 100 c.c. deprived of lead by sodium carbonate and the solution made up to 110 c.c.

50 c.c. of filtrate gave CuO = *nil.*
Polarisation in 400 mm. tube = $-0·130°$.

This laevo-rotation is very considerable, representing about 7 °/ₒ of the actual rotation usually measured in an analysis of leaf material. It is probably due to the presence of a gum which forms a lead salt which is not absolutely insoluble in water, so that the solution after filtration from the bulk of the precipitate is saturated with it; on precipitation of the lead with sodium carbonate, a soluble sodium salt is formed which is laevo-rotatory.

That this is the case appears on evaporating 300 c.c. of the 500 c.c. of clear solution obtained after precipitating with basic lead, to about 40 c.c. when a considerable quantity of gummy matter separates; the solution was filtered, washed to about 70 c.c. and sodium carbonate added so as exactly to precipitate the lead. The solution was filtered, evaporated to a syrup, which was then taken up with 50 c.c. of 95 °/ₒ alcohol, the extract filtered from the residue of gum, sodium acetate, etc., and the alcohol evaporated; the residue was dissolved in water and made up to 100 c.c.

50·00 c.c. gave CuO = *nil.*
Polarisation = $-0·130°$ in 400 mm. tube at 20°.

In this case the treatment with alcohol has removed approximately two-thirds of the gummy matter (bearing in mind that 300 c.c. of the original solution was taken); it is however extremely difficult, if not impossible, completely to eliminate the gum in this way by repeated treatment with alcohol, even when followed by addition of alumina cream to the aqueous solution finally obtained. The final solution always shows more or less laevo-rotatory power.

In working with leaf and plant material it is generally possible completely to extract the disturbing gummy material prior to the starch

[1] That this was the case was found not only by analysis but by microscopical examination by the chloral hydrate-iodine method; even the guard cells were free from starch.

conversion by a preliminary treatment with water. Thus in the case of the mangold leaf. by adding 200 c.c. of water and 5 c.c. of toluene to the leaf material and extracting for 24 hours at 38°, decanting and washing with a little water and subsequently converting with taka-diastase, in the ordinary way, a solution is finally obtained (after the usual treatment with basic lead and sodium carbonate) which in a 400 mm. tube shows a laevo-rotation of not more than 0·01°. It is noteworthy that the preliminary treatment with water fails to remove the greater part of the material precipitable by basic lead acetate, so that this treatment is necessary even after the preliminary extraction with water.

In the case of plant material from which gummy matter is extracted with extreme difficulty, it would probably be sufficient to introduce a correction for any active substances present by carrying out a control experiment or "blank" in which the diastase is omitted but the material otherwise treated exactly as in the actual estimation of starch.

The application of taka-diastase to the estimation of starch in cereals such as wheat, barley, etc., which present special difficulties, is being studied.

Appendix.

The ordinary diastase method and the error caused by the co-precipitation of dextrin.

To ascertain the degree of accuracy of this method under the most favourable conditions, a series of analyses were made, using purified potato starch dried *in vacuo* at 120° (see p. 154) until constant in weight; it was then gelatinised by heating with 50 c.c. of water in a boiling water bath during 15 minutes, cooled to 55° and subjected to the action of the diastase (0·100 grm. of the alcohol-precipitated enzyme) until all the starch was converted. This was generally the case after two or three hours. The diastase was then destroyed by heating the solution in boiling water during 15 minutes and the solution made up to 250 c.c. at 15°. The reducing power was estimated in 25 c.c. under Brown, Morris and Millar conditions and the rotatory power determined in a 400 mm. tube, the values assumed being maltose $[\alpha]_D = 138°$, dextrin $[\alpha]_D = 202°$.

TABLE VI. *Purified Potato Starch, Diastase Method.*

Conditions	Air dried starch taken	Weight dried in vacuo at 120°	% water in starch	CuO from 25 c.c. *	α_D in 400 mm. tube	Maltose total	Dextrin total	Starch found	% starch in vacuum dried material
5 hours	2·5017	1·9958	20·22	0·2211	5·090°	1·619	0·469	2·004	100·4
4 ,,	2·5110	2·0031	20·22	0·2191	5·125	1·604	0·4895	2·0095	100·3
5½ ,,	—	2·0732	—	0·2273	5·246	1·664	0·486	2·066	99·7
4 ,,	2·5110	2·003	20·22	0·2180	5·125	1·596	0·495	2·008	100·25
23 ,,	2·5002	1·9942	20·24	0·2401	4·968	1·758	0·3360	2·003	100·4
23 ,,	2·5002	1·994	20·24	0·2347	4·968	1·718	0·3636	1·992	99·9
15½ ,,	2·5118	2·0042	20·21	0·2291	5·040	1·677	0·414	2·003	100·0
								Average	100·1
Malt extract about 5 c.c. Made to 100 c.c.	3·0163	2·4220	19·70	0·2727 (ex. 10 c.c.)	15·24	2·000	0·5190	2·414	99·6

* The CuO in all cases is the average of two closely concordant values. Allowance has been always made for the reduction and polarisation due to diastase or malt extract; this was ascertained by a control experiment carried out under exactly the same conditions as in the corresponding experiment with starch. The experiment with malt extract was made on a different sample of starch.

These results (Table VI) are moderately satisfactory, but the following experiments (Tables VII to X) show that a great loss of dextrin occurs in the ordinary diastase method when any kind of precipitate is produced in the solution obtained from the hydrolysis; in these experiments basic lead acetate was added in different proportions and the lead was subsequently precipitated by sodium carbonate or hydrogen sulphide. The amount of dextrin removed depends on the quantity of lead precipitated, so that the lowest results for starch were obtained in the experiments in which the largest quantity of lead was used.

A. *Precipitation of the excess of lead by Sodium Carbonate.*

The starch was gelatinised with 50 c.c. of water and hydrolysed by diastase in the usual way at 55°; after conversion, the solution was washed into a 100 c.c. flask, and 5 c.c. of basic lead acetate added. Sodium carbonate was then added to precipitate the lead exactly and the solution diluted to 100 c.c.; it was then filtered and 10 c.c. used for reduction.

TABLE VII.

Air dried starch taken	Weight after drying in vacuo at 120°	% water in starch	CuO from 10 c.c.*	Maltose in 100 c.c.	a_D in 200 mm. tube	Dextrin in 100 c.c.	Total starch	% starch found in vacuum dried material
—	2·1843	—	0·2375	1·7395	5·42°	0·1550	1·804	**82·6**
2·0361	1·8601	19·34	0·1922	1·405	4·41	0·1329	1·464	**78·7**

B. *Precipitation of the excess of lead by Hydrogen Sulphide.*

1. *Using 5 c.c. basic lead acetate.* Procedure as in A, but the lead added to the starch conversion was removed by passing hydrogen sulphide through the solution after diluting to about 100 c.c. The solution was filtered and the precipitate thoroughly washed until the washings amounted exactly to 200 c.c. 20 c.c. used for reduction.

TABLE VIII.

Starch taken	Weight after drying in vacuo at 120°	% water	CuO from 20 c.c.*	Maltose in 200 c.c.	a_D in 200 mm. tube	Dextrin in 200 c.c.	Total starch	% starch in vacuum dried material
2·5008	2·0175	19·32	0·2002	1·464	2·69°	0·3316	1·719	**85·23**
2·5004	2·0162	19·36	0·2036	1·489	2·69	0·3158	1·727	**85·70**
2·4962	2·0132	19·38	0·2115	1·547	2·71	0·2862	1·753	**87·0**

2. Using 5 c.c. basic lead as in 1, but washing the precipitate of lead sulphide more thoroughly, viz. to 250 c.c., so as to ensure that the low results were not due to insufficient washing. 25 c.c. used for reduction.

TABLE IX.

Starch taken	Weight after drying in vacuo at 120°	% water	CuO from 25 c.c.	Maltose in 250 c.c.	a_D in 200 mm. tube	Dextrin in 250 c.c.	Total starch	% starch found in vacuum dried material
2·4783	1·9948	19·42	0·1874	1·376	2·05°	0·3285	1·634	**81·89**

3. Using 2 c.c. basic lead only; remaining procedure as in 1. Washing to 200 c.c.; 20 c.c. used for reduction.

TABLE X.

Starch taken	Weight after drying in vacuo at 120°	°/o water	CuO from 20 c.c.	Maltose in 200 c.c.	α_D in 200 mm tube	Dextrin in 200 c.c.	Total starch	°/o starch found in vacuum dried material
2·4990	2·0157	19·33	0·2080	1·5215	3·045	0·4672	1·9092	**94·7**
2·4950	2·0130	19·32	0·2087	1·5260	3·02	0·4504	1·898	**94·3**

TABLE XI. *Results obtained with purified Potato Starch (vacuum dried) by the modified Sachsse method (A.O.A.C.).*

Weight of starch dried in vacuo at 120—130° till constant	CuO ex 25 c.c.	Dextrose in 25 c.c.	°/o starch found
2·0239	0·2684	0·1059	**94·2**
2·0239	0·2680	0·1058	**94·1**
2·0187	0·2680	0·1058	**94·3**
2·0187	0·2666	0·1052	**93·8**
		Average......	**94·1**

In this method the material is heated during $2\frac{1}{2}$ hours with 200 c.c of water and 20 c.c. of hydrochloric acid of sp. gr. 1·125, so that hydrolysis is actually effected by 2·52 per cent. hydrochoric acid. After heating, the solution is neutralised with sodium hydroxide, made to 250 c.c. and the dextrose in 25 c.c. estimated by means of Fehling solution.

The values given show that the modified Sachsse method gives results which are 6 per cent. low as compared with those obtained by using diastase.

SUMMARY.

1. The Sachsse method of estimating starch is unreliable in the case of plant material; not only does the presence of pentosans falsify the results as pentoses are formed during the hydrolysis, but actual destruction of dextrose occurs during the prolonged treatment with dilute acid.

2. O'Sullivan's method gives low results owing to the loss of dextrin which occurs during the purification of the solution after the conversion by diastase.

3. A method is described for estimating starch based on the use of taka-diastase; under suitable conditions this converts the starch into maltose and dextrose only and no loss of these sugars occurs when the solution is treated with clearing agents such as basic lead acetate.

4. The necessity of removing substances soluble in water, such as gums, etc., which are optically active and thus cause error in the estimation of starch in plant material is emphasised. Special care is also necessary in sampling.

ENQUIRY INTO THE FACTORS WHICH CONTROL THE TEXTURE OF CHEDDAR CHEESE. PART I.

By ARTHUR GEAKE, M.Sc.

(From the Bio-Chemical Laboratory, Chemical Department, University of Bristol.)

VERY little work has yet been carried out on the Texture of Cheese although this is one of the most important factors controlling its market value. The present investigation was undertaken with the view of ascertaining the influence of "acidity" on curd, caseinogen and casein.

The effect which the nature of the surrounding liquid may have on the texture of a solid of a protein character was shown by Wood[1] for the case of wheat gluten. It seemed probable that the presence of acids, salts, etc. in whey might have an equally great effect on the texture of cheese curd.

According to Laxa[2], lactates of caseinogen containing more than 1 % lactic acid are soluble in water whilst those containing less than that amount are insoluble. Van Dam[3] found that in the presence of excess of lactic acid, caseinogen combined with 4·25 % of its weight of lactic acid, the resulting compound being soluble in water. On the other hand van Slyke and Hart[4] consider that the compound precipitated by the spontaneous souring of milk is caseinogen "dilactate," while a "monolactate" may be prepared by the addition of 32·6 grams of lactic acid to 20 lbs. of milk at 37—40° C. The chief constituent of American Cheddar Cheese they consider to be casein dilactate. Laxa[5] finds that by the dialysis of a solution of caseinogen in lactic acid a salt containing 1·4—1·9 % lactic acid is obtained, whereas by salting out from a solution in excess of lactic acid he obtains a compound containing 7·5 % acid. These two salts he regards as identical with the so-called mono- and dilactates of van Slyke and Hart.

[1] This *Journal*, II. 267 (1907–8).

[2] *Milchwirtsch. Zentralbl.* I. 538.

[3] *Chem. Weekblad.* I. 1013–9 (1910).

[4] *Amer. Chem. Journ.*, **32**, p. 154 (1904).

[5] *q.v.*

A second factor which must affect the texture is the absorption of acids by the curd. By following closely the variations in the acidity of the whey during the formation of a Cheddar cheese three distinct phases are noticed, viz.:

(1) A rise in acidity.
(2) A slight fall just when the coagulation sets in.
(3) A distinct rise.

It is remarkable that during the second phase the biuret reaction seems to become weaker, and may, in fact, disappear entirely.

The present paper can only deal with a small portion of this complicated subject and we therefore refrain, at present, from making deductions from the results obtained.

Experimental.

In the course of the investigation the following acids have been used: Fatty acids; formic, acetic, propionic, butyric, isobutyric, valerianic, and isovalerianic acids; oxy-acids; glycollic, lactic, α-oxybutyric, β-oxybutyric and oxy-isobutyric acids. With the exception of formic and acetic acids these were obtained in as pure a state as possible from Kahlbaum. The formic and acetic acids used were the ordinary laboratory reagents.

Part I. Solution of Pure Caseinogen and Casein in Acids.

Caseinogen. The caseinogen used was Kahlbaum's pure "Casein nach Hammarsten" and was dried to constant weight in an air-bath at 70° C. Analysis of the undried caseinogen gave the following results:

Water = 11·30 %. Ash = 0·26 %.
Nitrogen (by Kjeldahl's method) = 15·54, 15·76, 15·61, 15·54 %.
Mean 15·61 % N in the water- and ash-free caseinogen.

Casein. Casein was prepared both from caseinogen and directly from milk. In the first case rennet was allowed to act on a solution of caseinogen in disodium hydrogen phosphate and the casein thus formed precipitated by 10 % calcium chloride solution and purified by Hammarsten's method. After washing with alcohol and ether the pure casein was dried at 75—80° C. in an air-oven. A portion gave on analysis:

Water = 0·89 %. Ash = 0·51 %.
Nitrogen (by Kjeldahl) = 15·75, 15·33, 15·53 %.
Mean = 15·54 % in water- and ash-free casein.

From milk casein was prepared in the usual way. After coagulation with rennet the enzyme was destroyed by momentarily heating the curd and mother liquor to 90° C. with steam. The whole was poured into a large volume of water and the casein purified by Hammarsten's method. Analysis of the air-dried protein gave:

Water = $\begin{Bmatrix} 11·41 \\ 11·46 \end{Bmatrix}$ = 11·44 %. Ash = 1·03 %.

Nitrogen (by Kjeldahl's method) = $\begin{Bmatrix} 15·59 \\ 15·65 \end{Bmatrix}$ = 15·62 % in water- and ash-free casein.

It may be remarked here that there is a considerable lack of agreement as to the nitrogen content of casein. According to Köster[1], casein contains 15·84 % N. Rose and Schulze[2] however found 15·14 % N, whilst later Raudnitz[3] obtained 15·5 % N. The author's results are practically in agreement with those of the last-named worker, that the nitrogen content of casein does not differ noticeably from that of caseinogen. Since this work was carried out van Slyke and Bosworth[4] have obtained 15·80 % nitrogen in both casein and caseinogen.

Solution of Caseinogen and Casein in Fatty and Oxy-fatty Acids.

The solution of caseinogen and casein in the acids mentioned above was studied by the following method. A known weight of caseinogen or casein was added to 50 c.c. of a standard (N/10 or N/50) solution of the acid. After allowing to stand in a thermostat at 37—38° C. for the requisite length of time the solution was filtered through asbestos and the nitrogen content of a known volume of the filtrate estimated by Kjeldahl's method. The weight of caseinogen or casein was obtained from that of nitrogen by multiplication by the factor 6·37. To prevent putrefaction a few drops of toluene were added in each case.

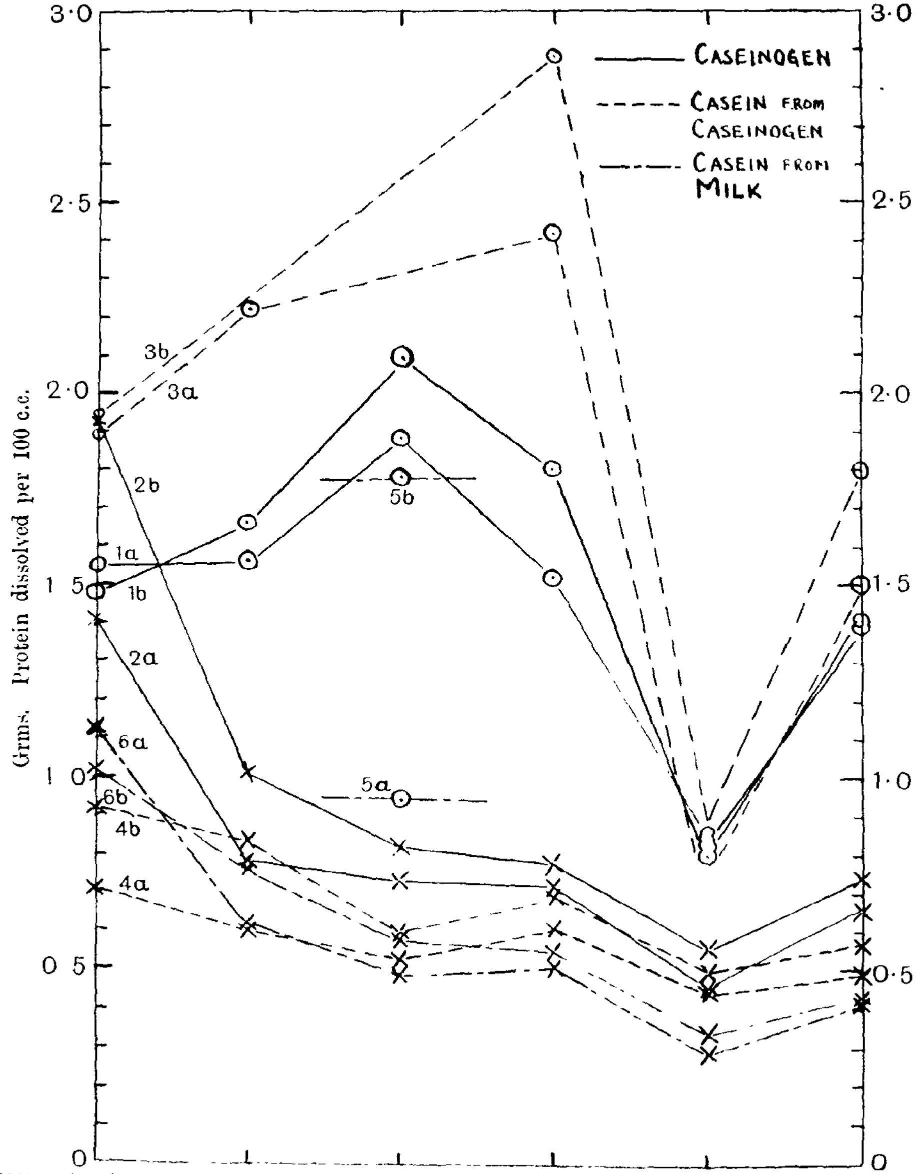
Preliminary experiments with caseinogen and lactic acid showed that the weight of caseinogen dissolved was dependent on the amount present. Thus, though in the presence of a large excess of caseinogen

[1] *Biol. Centralbl.* **2**, No. 2; Maly's *Jahresberichte*, **11**, 14 (1881).

[2] *Landwirtsch. Versuchsstationen*, **31**, 115 (1885); Abderhalden's *Biochemisches Handlexicon*, IV. (1), p. 118.

[3] *Monatshefte f. Kinderheilk.* **2** (1904); Abderhalden's *Biochem. Handlexicon*, IV. (1), p. 118.

[4] *J. Biol. Chem.* **14**, 203 (1913).



N/10 Acetic Propionic Butyric Isobutyric Valerianic Isovalerianic
for curves marked × (2 *a*, 2 *b*, 4 *a*, 4 *b*, 6 *a*, 6 *b*).

N/50 Formic Glycollic Lactic *a*-Oxybutyric *β*-Oxybutyric Oxy-isobutyric
for curves marked ⊙ (1 *a*, 1 *b*, 3 *a*, 3 *b*, 5 *a*, 5 *b*).

FIG. 1.

100 c.c. of N/50 lactic acid is capable of dissolving at least $1\frac{1}{2}$ grams, yet when only 0·2 gram of caseinogen was present a distinct residue could still be seen after the lapse of a week. Curves 1 and 2 in Fig. 2 B show the weights of caseinogen dissolved when the amounts shown by the ordinates were added to 100 c.c. N/50 lactic acid. Curve 1 gives the results after 17 hrs. and 2 after 170 hrs. It will be seen that if sufficient time is allowed a maximum is reached in the presence of a large excess of caseinogen. It is evident that the process can be neither one of simple solution nor of simple reaction, nor of the two combined. As is well known caseinogen in the presence of this and other solvents swells before solution, and it seems probable that it is this swelling that is responsible for the above results.

In the subsequent experiments a large excess of the protein was used and the weight dissolved estimated after 1 and 2 weeks. In the cases of the oxy-acids and of formic acid 5 grams of caseinogen or casein were added to 50 c.c. of N/50 solutions of the acids, whilst with the fatty acids, which are much less powerful solvents, 2 grams of the protein were added to 50 c.c. of N/10 solutions. The results are shown in Fig. 1 calculated for 100 c.c. of solvent. Formic acid has been treated as an oxy-acid as it behaves like them in this respect. In the curves marked "*a*" the experiments were interrupted after 1 week, in those marked "*b*" after 2 weeks.

The more powerful solvent action of the oxy-acids is probably due to their greater strength as acids. This also accounts for the powerful solvent action of formic acid since this acid is even stronger than the oxy-fatty acids. Casein is somewhat less soluble in fatty acids than caseinogen but in most cases more soluble in the oxy-fatty acids.

Part II. The Solution of Curds in Lactic and Acetic Acids.

It has been shown above that in the presence of weak organic acids, caseinogen and casein swell and finally dissolve to a greater or lesser extent. It was now desired to discover whether the protein matter would be equally readily removed from an ordinary cheese curd and whether the other substances present in the curd would protect the protein from the action of the acid. Curds were therefore prepared from milk both by the action of rennet and of acid and the solvent action of lactic and acetic acids studied.

Preparation of Curds: Rennet Curds.

For duplicate experiments two separate curds were prepared as follows. About 1700 c.c. of fresh milk were heated in a thermostat to about 32° C. and treated with sufficient rennet powder to cause coagulation. The curd was allowed to stand till it contracted together, broken up to assist the removal of whey and pressed for several hours in a small screw-press. The details were as under.

	I	II
Milk.................... ..	1630 c.c.	1730 c.c.
Rennet powder	0·5 grm.	0·1 grm.
Temperature	32° C.	—
Coagulation time	2—3 mins.	18 mins.
Weight of curd..... ..	205 grms.	212 grms.

I. Dried in vacuo over sulphuric acid to constant weight:

$$\text{Water} = \begin{Bmatrix} 53{\cdot}59 \\ 53{\cdot}64 \end{Bmatrix} = 53{\cdot}62\ \%.$$

Extracted in a Soxhlet with ether:

$$\begin{aligned} \text{Fat} &= 39{\cdot}21\ \% \text{ of dry curd,} \\ &= 18{\cdot}19\ \% \text{ undried curd.} \end{aligned}$$

II. Dried in vacuo at 40° C. over sulphuric acid to constant weight:

$$\text{Water} = \begin{Bmatrix} 57{\cdot}66 \\ 58{\cdot}50 \end{Bmatrix} = 58{\cdot}08\ \%.$$

Acid Curd.

2270 c.c. of fresh milk was diluted to 5 times its volume and coagulated by the addition of glacial acetic acid to a concentration of 0·2 %. After the curd had been allowed to settle the supernatant liquid was syphoned off and the curd washed by decantation with distilled water. It was collected on a cloth and pressed as dry as possible in a small screw-press. The material thus obtained was less coherent and softer than the rennet curd. Weight, 255 grams.

Dried in vacuo at 40° C. over sulphuric acid and to constant weight:

$$\text{Water} = \begin{Bmatrix} 53{\cdot}24 \\ 51{\cdot}54 \end{Bmatrix} = 52{\cdot}39\ \%.$$

Solution of Curds in Acids.

About 35 grams of the undried curd were added in large pieces to 150 c.c. of water, N/50, N/100 and N/200 lactic acid and N/10 acetic acid and kept in stoppered bottles at 37—38° C. From time to time 10 c.c. of the supernatant liquid were withdrawn for a Kjeldahl estimation.

Entry of undissolved particles into the pipette was prevented by covering the point with a cotton-wool filter. From the weight of nitrogen found the protein per 100 c.c. solution was calculated. To prevent putrefaction an excess of toluene was always added. In spite of this the rennet curds frequently became putrid after about a week, which must be ascribed to the action of the rennet still in the curd. The acid curds always remained fresh. In the table the results are calculated for 100 c.c. of solvent.

TABLE I.

Time	Protein in 100 c.c. water	Excess dissolved by 100 c.c.			
		N/50 Lactic acid	N/100 Lactic acid	N/200 Lactic acid	N/10 Acetic acid
hours	g.	g.	g.	g.	g.
Rennet Curd I					
18	0·216	−0·012	+0·002	+0·024	−0·004
48	0·504	−0·062	−0·073	+0·139	−0·105
167	1·79	+0·56	−0·29	-	−0·63
	(Putrid)	(Fresh)	(Slightly putrid)	(Putrid)	(Fresh)
Rennet Curd II					
18½	0·240	−0·021	−0·037	−0·023	−0·062
48	0·423	−0·013	−0·005	+0·018	−0·098
96	0·651	−0·049	+0·011	−0·013	−0·143
186	1·82	−0·70	−0·19	−0·38	−0·48
	(Putrid)	(Fresh)	(Putrid)	(Putrid)	(Fresh)
Acid Curd					
25	0·109	0·473	—	0·123	0·289
73	0·246	0·774	0·189	0·075	0·456
121	0·353	0·817	0·197	0·069	0·450
217	0·446	0·874	0·198	0·064	0·491

It will be seen from the table that the presence of lactic or acetic acids hinders the solution of rennet curd. This is probably due to the acids hindering the action of the enzymes since these solutions did not become putrid so readily as those in pure water. The acid curd, in which the presence of large amounts of enzymes was excluded, was much more readily dissolved by the acids than by water and by the stronger than by the weaker lactic acid solutions. The weight of nitrogen dissolved by pure water was still increasing after nine days, but the excess over this weight caused by the addition of acid became

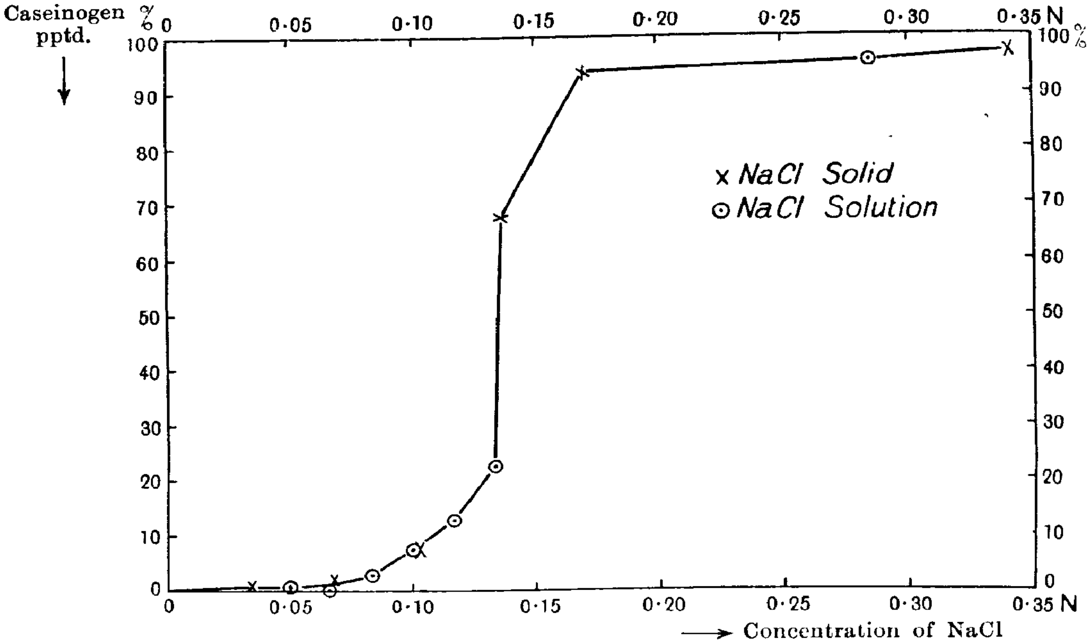

Caseinogen % pptd.
0 0·05 0·10 0·15 0·20 0·25 0·30 0·35 N
100 90 80 70 60 50 40 30 20 10 0
100 %
× NaCl Solid
⊙ NaCl Solution
0 0·05 0·10 0·15 0·20 0·25 0·30 0·35 N
Concentration of NaCl

FIG. 2 A.

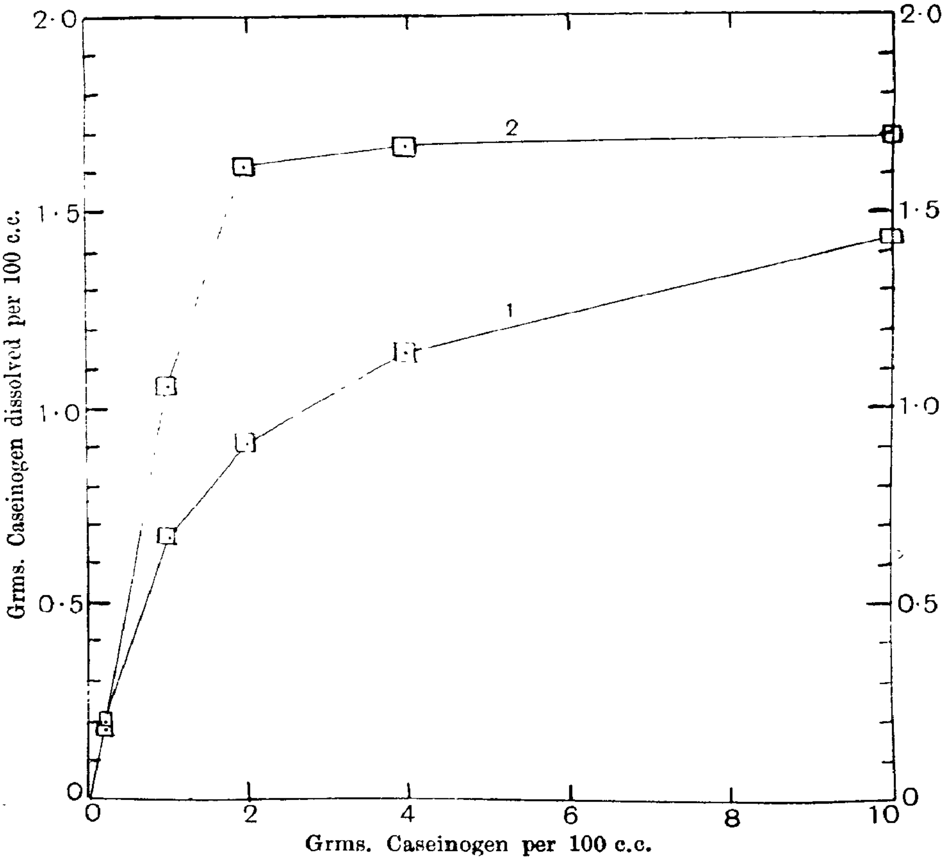

2·0 1·5 1·0 0·5 0
Grms. Caseinogen dissolved per 100 c.c.
2
1
0 2 4 6 8 10
Grms. Caseinogen per 100 c.c.

FIG. 2 B.

practically constant in a few days. The total weights of caseinogen dissolved from the curd by 100 c.c. N/50 lactic acid (1·320 g.) and by 100 c.c. N/10 acetic acid (0·937 g.) are considerably less than those dissolved in the presence of the pure protein (as given in Fig. 1), but this is probably due to insufficient time being allowed for solution to take place from the interior of the curd. Only in a few cases did any pronounced separation of fat take place though the temperature was, of course, above its melting point.

Part III. Precipitation of Caseinogen by Sodium Chloride.

The addition of sodium chloride to lactic acid solutions of caseinogen lessens the solubility of the caseinogen, and if sufficient salt is added the protein is more or less completely salted out. As in the case of the solution of caseinogen in lactic acid so in the case of this precipitation the process is not a simple one since it becomes more complete on prolonged standing.

Thus in one experiment 4 c.c. N/10 lactic acid and 16 c.c. of a normal solution of sodium chloride in N/10 lactic acid were added to 100 c.c. of a solution of caseinogen in N/10 lactic acid containing 3·89 grams caseinogen per 100 c.c. The mixture was allowed to stand at room temperature and from time to time portions were filtered off, and the nitrogen estimated in a known volume of the filtrate. The weights of caseinogen precipitated could then be calculated. The results in the table below have been calculated for 100 c.c. of solvent, and the amount of caseinogen precipitated expressed as a percentage of the total caseinogen.

TABLE II.

After mixing, caseinogen = 3·24 g. per 100 c.c.

Time hours	Caseinogen in solution g.	Caseinogen pptd. g.	Caseinogen pptd. %
0	3·08	0·16	4·9
0·33	3·03	0·21	6·5
0·67	2·83	0·41	12·7
1·00	2·67	0·57	17·6
24	0·42	2·82	87·0

In the following measurements of the weight of caseinogen precipitated from lactic acid solutions by different concentrations of salt the

solutions were rapidly filtered through Gooch asbestos under pressure after adding the salt and shaking for a few seconds. The caseinogen was always dissolved in N/10 lactic acid. The sodium chloride (Kahlbaum's pure) was added either as the finely powdered crystals or in solution in N/10 lactic acid; as will be seen from the curve the end result is the same in either case. The mixture was always N/10 with respect to lactic acid. After filtration a Kjeldahl estimation was made of a known volume of the filtrate. In Fig. 2 A the results are shown in percentages of the total caseinogen precipitated. The original concentration of caseinogen varied between 2·76 and 4·17 grams per 100 c.c.; it appears to have no influence on the result. The most striking feature of the curve is the very rapid change in the percentage of caseinogen precipitated by concentrations of sodium chloride between about 0·10 N and 0·17 N. At the former concentration the amount of caseinogen precipitated is less than 10 %, and at the latter it is more than 90 %. This illustrates the value of the method of separating proteins by fractional salting out from solution. It is probable that curves of the same type would be obtained for other cases of salting out.

In conclusion I wish to express my thanks to the Colston Research Committee who have substantially helped the investigation by several grants towards the expenses.

A CASE OF CORRELATION IN WHEAT.

By W. H. PARKER, B.A.

(Plant Breeding Institute, School of Agriculture, Cambridge.)

WHILE investigations were being carried out concerning the question of variability within a variety, the case of correlation described below was observed.

The material used was a sample of Squareheads Master wheat purchased from Messrs Webb, of Stourbridge. The grain was sown in the autumn of 1912, and before harvest every plant which was not, without doubt, true Squareheads Master was discarded.

The character investigated was that of the density of the ear, and to obtain complete accuracy the method employed by Nilsson-Ehle in his investigations on the inheritance of that character was employed. This method was used by him in the investigations described in the chapter on "Internode-length of Wheat" in his *Kreuzungsuntersuchungen an Hafer und Weizen*[1]. The density of the ear is judged from the average length of its internodes. The method used is merely to measure the total length of the rachis in millimetres and to divide it by the total number of internodes, the quotient, thus, being the average internode-length for the ear.

While using this method for estimating the average internode-length of the main tiller of each of 1887 plants of Squareheads Master, the author was struck by the fact that there appeared to be high *correlation between the total length of the rachis and the average internode-length.* With the help of Mr G. Udny Yule the following Correlation Table was compiled, and the coefficient calculated. As will be seen, the coefficient of correlation proved to be $+0{\cdot}9099$, that is to say that the correlation is very high.

In this case $I = 0{\cdot}998 + 0{\cdot}0303\,L$. "I" being estimated internode-length, and "L" is the given rachis-length.

[1] Part II. p. 26 *et seq.*: *Lunds Universitets Årsskrift N.F.*, Afd. 2, Bd. 5, Nr. 2.

SQUAREHEADS MASTER, 1913.

Correlation Table between Internode-length in mm. and Rachis-length in mm.

Internode-length

Total Rachis-length	2·5	2·6	2·7	2·8	2·9	3·0	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9	4·0	4·1	4·2	4·3	4·4	4·5	4·6	4·7	4·8	4·9	5·0	Total
49—50			1																								1
51—52							1																				1
53—54			1	1																							2
55—56	1	2	1	2	1	—	1																				8
57—58		1	2	5	1																						9
59—60		1	5	1	1	2																					10
61—62		1	2	5	6	4	6	—	—	2																	26
63—64			2	—	14	5	6	2	1																		30
65—66				7	3	15	9	2	2	—	1																39
67—68				2	7	15	13	8	—	3	—	1															49
69—70					8	26	15	10	9	—	3	3															74
71—72					1	21	45	8	7	8																	90
73—74						2	10	52	16	12	1	—	2														95
75—76						5	19	28	72	12	12	4															152
77—78							10	26	39	43	13	8	2														141
79—80								14	77	30	43	15	—	2													181
81—82							2	6	8	76	32	28	13	—	1	—	2										168
83—84								3	10	12	96	23	20	3	—	1											168
85—86									2	30	36	35	25	3	4	—	1										136
87—88									1	1	33	26	29	15	1	2	—	1	—	1							110
89—90										2	—	32	24	23	19	2	—	—	1								103
91—92											2	13	18	41	—	8	1	—	—	—	1						84
93—94												1	13	11	23	3	2	1									54
95—96													2	23	1	14	2	2	1								45
97—98													2	4	6	5	10										27
99—100														1	1	14	2	1	—	1							20
101—102															3	3	4	4	1	2							17
103—104																1	3	6	4								14
105—106																1	1	2	2	5							11
107—108																1	—	1	3	1	1						7
109—110																				1	1	1					3
111—112																			1	1							2
113—114																					2	—	1				3
115—116																						2					2
117—118																											0
119—120																						1	—	1			2
121—122																							1				1
123—124																									1	1	2
Total ...	1	5	14	23	42	95	137	159	244	231	272	189	150	126	59	55	28	18	13	12	5	4	2	1	1	1	1887

It may be worth mentioning that the correlation has been worked out for a few Pure Lines from this material, and has proved to be practically identical with that of the population.

Other varieties of wheat have not yet been investigated from the same point of view, but it may reasonably be hoped that a similar degree of correlation would be found, though the corresponding values of the two variables would certainly be different.

In consideration of the very small deviation of the actual internode-lengths in comparison with those estimated, the investigator would seem at present to be justified in utilising the more easily measured total rachis-length when working on questions of the density of ears within a variety of wheat. It is possible that the relation between the internode-length and the total rachis-length may prove to be the most satisfactory method of classifying wheats according to the density of their ears, as the practice of grouping them into classes by eye alone, as is done at Svalöf, is purely arbitrary, and, as will be seen by the correlation table here given, the internode-length, as used by Nilsson-Ehle, is too variable within a variety to be used for classification; the relation between internode-length and total rachis-length, on the other hand, can be estimated with great accuracy, and is, at least in this case, constant within a variety.

ON OVARIOTOMY IN SOWS; WITH OBSERVATIONS ON THE MAMMARY GLANDS AND THE INTERNAL GENITAL ORGANS.

By K. J. J. MACKENZIE, M.A., F. H. A. MARSHALL, Sc.D., and J. HAMMOND, M.A.

(*School of Agriculture, Cambridge.*)

PART III.

In our previous papers[1] we have shown that the black pigment found in the mammary tissue of sows of various coloured breeds is related to the colour rather than to the sexual condition of the pig. We have also pointed out that this conclusion is contrary to the statements of bacon curers who rely on the presence of this pigment for an indication as to whether or not a sow is 'on heat.' Their method of testing the condition of the sow has been described by us in a former paper. It follows from the assumption made by the bacon curers that pigment should not be present in the 'belly pieces' of spayed sows (or to be strictly accurate, of sows spayed in early life, before the mammary ducts and glands have undergone development).

The point is of such great importance commercially, that it seemed desirable to us to accumulate more evidence concerning the whole question as to the conditions under which the black pigment of the mammary glands commonly occurs. This we were enabled to do through the kindness of Messrs C. and T. Harris & Co., Ltd. of Calne, Mr Reynolds of Devizes, and Mr Hasler of Dunmow, whose respective bacon factories we visited at a time when pigs in considerable numbers were being slaughtered.

The methods adopted by us at the Calne, Devizes, and Dunmow factories were as follows. One of us examined all the pigs (at Calne as many as 124) just before being slaughtered, and noted, as far as it was possible to do so, their sexual condition (i.e. their sex, and in the

[1] This *Journal*, vol. IV. June 1912, and vol. V. October 1913.

case of the sows the stage in the oestrous cycle). The pigs were all numbered so that each one could be identified for the purposes of subsequently examining the internal generative organs and the mammary tissue. This was done in due course shortly after the killing. It was found possible from an examination of the ovaries and uterus to check roughly the observations on the living animals in regard to whether they were in a condition of heat or approaching heat. Thus largely protruding follicles, very recently ruptured follicles (showing that ovulation had taken place shortly before) or older corpora lutea were duly noted. The degree of congestion of the uterus was also observed. At a later stage the 'belly pieces' were cut into in order to see whether pigment was present, in exact accordance with the method carried out in ordinary practice.

The following table represents the results arrived at, coloured pigs only being included, since pigment of the kind referred to was never found to occur in animals belonging to any of the white breeds. The table includes 124 pigs at Calne, 79 killed at Devizes, 103 at Dunmow, besides 11 pigs killed at Cambridge.

TABLE I. *Occurrence of mammary pigment in relation to sexual state of pig.*

	Black			Black and White		
	Pigmented	Non-pigmented	°/ₒ pigmented of pigs examined	Pigmented	Non-pigmented	°/ₒ pigmented of pigs examined
Hogs	12	30	28·6	0	72	0
Normal sows	15	4	78·9	8	34	19·0
Spayed sows	6	0	100·0	2	35	5·4
Sows probably about the heat period	8	2	80·0	3	6	33·3
Sows on heat	3	3	50·0	1	2	33·3
Pregnant sows	0	0	—	2	0	100·0
Old sows	0	7	0	0	0	—

The two sows containing foetuses had probably been pregnant about a fortnight.

It was evident that the occurrence of pro-oestrum or oestrus was in no way correlated with the presence or absence of pigment in the mammary area. Neither could it be said that pigment was present in greater quantity during the heat period.

It is shown also that pigment was present in many of the spayed sows. There were, however, some slight indications that the amount present was liable to be less in the operated pigs. This result (which is by no means firmly established) would not be altogether surprising in view of the fact that the pigment always occurs in close relation to the mammary ducts and glands, and that these are usually less developed in the spayed sows than in open ones. It must be remembered, however, that the degree of development of the mammary tissue is not very great in sows which have never been pregnant, since a true mammary hypertrophy only takes place concurrently with gestation. Nevertheless, the distribution of the pigment in spayed and other non-pregnant sows is often quite considerable enough to discolour almost the whole of the belly piece.

In the seven old sows no mammary pigment was found. This may possibly be accounted for by the assumption that the pigment is broken down or absorbed during the stage of glandular activity. However, more evidence is required before this point can be determined.

In all the hog pigs in which mammary pigment occurred, it was limited to a small area round the base of each nipple, this probably corresponding to an adventitious duct growth, such as is frequently found in male animals.

The table which follows illustrates the occurrence of mammary pigment in relation to the colour of the pig. Berkshire pigs are classed as black and white.

TABLE II. *Occurrence of mammary pigment in relation to colour of pig.*

	Pigmented	Non-pigmented	% pigmented of pigs examined
Black			
Hogs	12	30	28·6
Sows	32	16	66·6
Black and White			
Hogs	0	72	0
Sows	16	77	17·2
White			
Hogs	0	27	0
Sows	0	35	0
Red (Tamworth)			
Sows	1	0	100·0

As already mentioned the pigment does not occur in white pigs. It is commonest in the black sows, but occasionally is found also in black hogs. In black and white sows it is not quite so frequent as in black sows, and there is some evidence that, when present, it occurs only where there is a black patch on the skin. Thus in black and white sows, pigment is present in some of the mammary glands, but absent in others, according to the colour of the surrounding skin. But apart from this relation we found a considerable amount of variation in the quantity and distribution of the mammary pigment in the coloured breeds, and in black pigs it sometimes happened that some of the glands were pigmented while others were not. It is possible that in such cases the absence of pigment from some of the glands was due to the ducts not having developed, or to past phases of activity undergone by certain of the glands, whereby they had lost their pigment in the manner suggested above for old sows which had been pregnant.

The case of the Red Tamworth sow affords an interesting confirmation of the view that the mammary pigment is derived from the skin pigment. On cutting open the belly, the tissue in the region of the mammary glands was found to be pigmented; the colour of the pigment, however, was not black but sandy, of the same shade, but not so intensely coloured, as the hair. Pieces were preserved, and these on being cut into sections showed the characteristic pigment granules in the cells of the mammary ducts.

We take this opportunity to express our thanks to the proprietors of Messrs C. and T. Harris & Co., Ltd., the Central Wiltshire Bacon Co., Ltd., and the Dunmow Flitch Bacon Co., Ltd. for their courtesy in allowing us to carry out this investigation. We would further like to thank Messrs Beazley, A. E. Millar and H. H. Whitaker, officials of these companies, as well as their staffs for much assistance given to us while examining over three hundred pigs.

Further case of Incomplete Ovariotomy.

In a former paper we gave an account of two cases of incomplete ovariotomy in sows which were found to come on heat after they were supposed to have been completely de-sexed. Since these were described, a further case of a similar kind has been supplied by one of our own sows.

The sow was a cross bred Middle-Large White, and the operation by which she was supposed to have been spayed was performed on

March the 28th, 1913, when she was seven weeks and three days old. On June the 24th, when just under five months old, she was noticed to show distinct signs of oestrus, a condition which was repeatedly observed until her death. An examination of her internal generative organs, which had been preserved at the time of spaying, showed that these were much torn, and that one ovary was missing. After the sow was slaughtered the ovary was found *in situ*, apparently quite normal, and containing a large number of protruding Graafian follicles.

These cases show clearly, what had already been deduced from the study of the physiology of the generative organs in other animals, that heat only occurs in sows when functional ovarian tissue is present, and that removal of the uterus without the complete removal of both ovaries is totally ineffective in preventing the recurrence of the oestrous cycle.

We again acknowledge, with thanks, that the expenses of this investigation have been defrayed by a Special Research Grant of the Board of Agriculture and Fisheries from funds placed at their disposal for that purpose by the Development Commissioners.

OBSERVATIONS ON THE PERITHECIAL STAGE OF THE AMERICAN GOOSEBERRY-MILDEW (*SPHAEROTHECA MORS-UVAE* (*Schwein.*) *Berk.*).

By E. S. SALMON.

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So far as I am aware, the manner of the dehiscence of the perithecium of *Sphaerotheca mors-uvae* has not hitherto been described. Although in the main features the process of dehiscence is very similar to that which I have described for *Erysiphe Graminis*[1] and for *Sphaerotheca Humuli*[2], some new points of interest have been observed.

During the spraying experiments[3] against the American Gooseberry-mildew which were carried out last year in Kent, it was observed that the fully-developed winter-stage occurred on both the berries and young shoots of gooseberry bushes at the beginning of August. The perithecia, which were perfectly mature, readily separated from the persistent mycelium; indeed, a considerable number were already loose, as was shown by the fact that if infested berries were held over a white sheet of paper and gently tapped, the perithecia fell in dozens on to its surface. The separation of the perithecium from the mycelium at this early date is a point of considerable practical importance in the control of the disease, indicating as it does the absolute necessity for the collection and destruction of mildewed berries before the winter-stage has matured on them, as otherwise the soil will become infested. Perithecia from this material when supplied with moisture were found to dehisce and eject the ascospores within a few hours. It seems probable, therefore, that in some cases the ascospores of *S. mors-uvae* may serve to spread

[1] E. S. Salmon, in *Journal of Botany*, 1903, p. 161.

[2] Idem, in *Journ. of Agric. Science*, II. p. 329 (1907).

[3] An account of these appears in the *Journal of the Board of Agriculture*, March, 1914.

the disease in the same season in which they are produced,—as I have proved is sometimes the case with *E. Graminis.*

Similar material, showing the fully-developed winter-stage, was collected in November last, and it has been found that this, when kept dry in the laboratory (temperature about 15° C.) remains living through the winter. In February many of the perithecia when supplied with moisture opened and the ascus discharged its spores,—the whole process taking place within a few hours. The manner of the dehiscence of the perithecium and the swelling up of the ascus and discharge of the spores, as it takes place in a drop of water, can be seen on reference to Figs. 1 to 3, drawn under the microscope. A small more or less vertical slit appears at the apex of the perithecium, allowing at first only the tip of the enclosed ascus to be seen (see Fig. 1). The ascus rapidly absorbs water and swells considerably, emerging more and more into the open, and thereby exposing to view its ascospores. Through the forcible swelling of the ascus, the slit in the walls of the perithecium is enlarged. In about 5 minutes the ascus has swollen enormously, being now often double the length of what it was inside the perithecium (see Figs. 2, 4, 5). In the process of swelling its wall naturally becomes thinner and thinner; finally the tension becomes so great that the wall is ruptured near the apex of the ascus, at a place where from the first the wall is much thinner, forming there a kind of "pore" (see Fig. 9). Through the slit the 8 ascospores are forcibly shot out all together. I have seen them expelled through water to a distance of about ten times the diameter of the perithecium, and from a perithecium laid on wet filter paper the spores will be discharged to a distance of 2·5 cm. The ruptured and empty ascus, greatly reduced in size, shrinks back into the perithecium, the walls of which at the slit come nearer together (see Fig. 3). If, as sometimes happens when the perithecium is in water, the ascus gradually emerges entirely from the perithecium, the process of the discharge of the spores takes place in the same way.

The length of time taken by the ascus in discharging its spores (when the perithecium is immersed in water) varies, as the following observations show.

Obser. 1. *12.53 p.m.*, Perithecium dehisced; apex of swelling ascus just visible (as in Fig. 1). *12.54 p.m.*, ascus emerged sufficiently to show 3 ascospores. *12.55 p.m.*, 6 ascospores visible. *12.57 p.m.*, clear vacuole-like spaces (see below) now apparent round most of the spores. *1 p.m.*, 8 ascospores now visible; ascus fully extended (as in Fig. 2). *1.5 p.m.*, ascospores discharged.

Obser. 2. *3.43 p.m.*, Perithecium dehisced. *3.44 p.m.*, ascus emerging and showing 2 of the ascospores. *3.45 p.m.*, ascus further emerged, and showing 4 ascospores,

with clear vacuole-like spaces round two of them. *3.48 p.m.*, 8 ascospores visible, most of them surrounded by clear vacuole-like spaces. *4 p.m.*, ascus apparently fully extended, wall very thin. *4.29 p.m.*, ascospores discharged.

In a third case the length of time from the dehiscence to expulsion was 11 minutes.

The following facts are summarised from Exper. 2, given below in detail (see p. 190). Perithecia continued successively to dehisce over a period of 19 days, after variations of temperature from 15·5° C. to − 1° C. With a temperature as low as 4·5° to 3° C., and as high as 27° C., the perithecium dehisces and discharges its spores. A temperature of − 1° C., repeated five times, does not kill the ripe, or nearly ripe, perithecium.

In the following experiments the perithecia were kept on wet filter paper in a Petri dish, and placed so as to be about 5 mm. below the lid. The ascospores on being expelled were caught either in the drops of condensation on the lid or in agar, and were counted under the microscope and then removed after each observation.

Exper. 1. At 10.30 a.m. Feb. 12, material of the winter-stage (taken from bushes last November, and subsequently kept dry in the laboratory) was wetted and placed immediately on wet filter-paper in 3 Petri dishes. These were put in the following positions: (1) out-of-doors, in the shade. The maximum temperature during the day was 13° C.; (2) in the laboratory, temp. 15° to 16° C.; (3) in an incubator, temp. 27° C. By 12 noon the number of ascospores which had been discharged was as follows: (1) 16; (2) 45; (3) several hundreds. By 3 p.m. several hundreds had been discharged from (1) and (2).

Exper. 2. The dry material[1] was wetted at 11.15 a.m. on Feb. 13, and at 11.33 a.m. was placed in Petri dishes in the same positions as in *Exper.* 1. The number of ascospores *successively discharged*, and the variations of temperature, are shown in the following table (see p. 190).

The ascospores germinated normally after being kept for some hours in water.

The living ascus has the power of swelling and shrinking several times. If a current of a 2·5 or 5 °/₀ solution of common salt is drawn through water in which there is an ascus which has swollen out as shown in Fig. 2, the ascus shrinks very rapidly, and the progressive thickening of the wall in consequence can be clearly seen under the microscope. (Cf. Figs. 7, 8, 9.) If distilled water is now added, the ascus at once begins to enlarge again, the cell-wall becoming thinner and thinner, until the ascus attains its original volume. The process can be repeated

[1] To secure as far as possible uniformity of the material as regards degree of maturity, every patch of mycelium (with its perithecia) taken from the shoots was divided into three portions, and one piece placed in each Petri dish. This was easy to do, as the patches were from 5 to 8 mm. long. The total size of the material placed in each Petri dish was 1 × ·5 cm.

EXPERIMENT 2.

Time of examination	1		2		3	
	Temp. °C.	No. of spores	Temp. °C.	No. of spores	Temp. °C.	No. of spores
Feb. 13, 12.30 p.m.	10	0	14	0	27	8
,, 1.0 ,,	10·5	0	14·5	0	27	15
,, 2.0 ,,	11	12	15·5	20	27	98
,, 2.30 ,,	10·5	30	15·5	42	27	34
,, 3.30 ,,	10	21	15	42	27	67
,, 4.30 ,,	9	15	15	110	27	20
,, 5.30 ,,	9	34	15	35	27	5
,, 9.0 ,,	10	over 200	15	over 100	27	30
Feb. 14, 9 a.m.	10—7 *	bet. 500 & 600	15	about 200	27	0
,, 1 p.m.	12	about 200	17	8	27	10
,, 5 ,,	10	about 150	17	10	27	0
,, 10 ,,	11	18	17	0	27	0
Feb. 15, 9 a.m.	11—8·5	64	15	0	27	0
,, 2 p.m.	11	16	15	0	27	0
,, 9 ,,	7	0	15	0	27	0
Feb. 16, 9 a.m.	7—4	9	15	0	27	0
,, 9 p.m.	6·5	8	15	0	27	0
Feb. 17—18. Not observed	—	—	—	—	—	—
Feb. 19, 2 p.m.	***	about 30	15	0	27	0
,, 9 p.m.	6·5	8	15	0	27	0
Feb. 20, 9 a.m.	6·5—1	8	15	0	27	0
,, 9 p.m.	7·5	8	15	0	27	0
Feb. 21, 9 a.m.	9—5	8	15	0	27	0
,, 9 p.m.	9—4**	8	15	0	27	0
Feb. 22, 9 p.m.	—	16	15	0	27	0
Feb. 23, 9 a.m.	4·5—3	16	15	0	27	0
,, 9 p.m.	9—4·5	8	15	0	27	0
Feb. 24, 9 a.m.	6·5—−1	0	15	0	27	0
,, 9 p.m.	13—2	2	15	0	27	0
Feb. 25, 9 a.m.	4·5—3	0	15	0	27	0
,, 9 p.m.	12—2	3	15	0	27	0
Feb. 26, 9 a.m.	5·5—−1	0	15	0	27	0
,, 9 p.m.	9—0	0	15	0	27	0
Feb. 27, 9 a.m.	3·5—−1	0	15	0	27	0
,, 9 p.m.	15·5—−1	0	15	0	27	0
Feb. 28, 9 a.m.	5·5—0	0	15	0	27	0
,, 9 p.m.	13—3	8	15	0	27	0
March 1, 9 a.m.	8—2	0	15	0	27	0
,, 9 p.m.	14·5—4·5	0	15	0	27	0
March 2, 9 a.m.	5·5—·5	16	15	0	27	0
,, 9 p.m.	—	0	15	0	27	0
March 3, 9 a.m.	5·5—0	0	15	0	27	0
,, 9 p.m.	10·5—2	6	15	0	27	0

* Maximum and minimum readings between 9 p.m. and 9 a.m.
** ,, ,, ,, ,, ,, 9 a.m. and 9 p.m.
*** The max. and min. readings between Feb. 16, 9 p.m. and Feb. 19, 2 p.m., was 10° C. and −1° C.

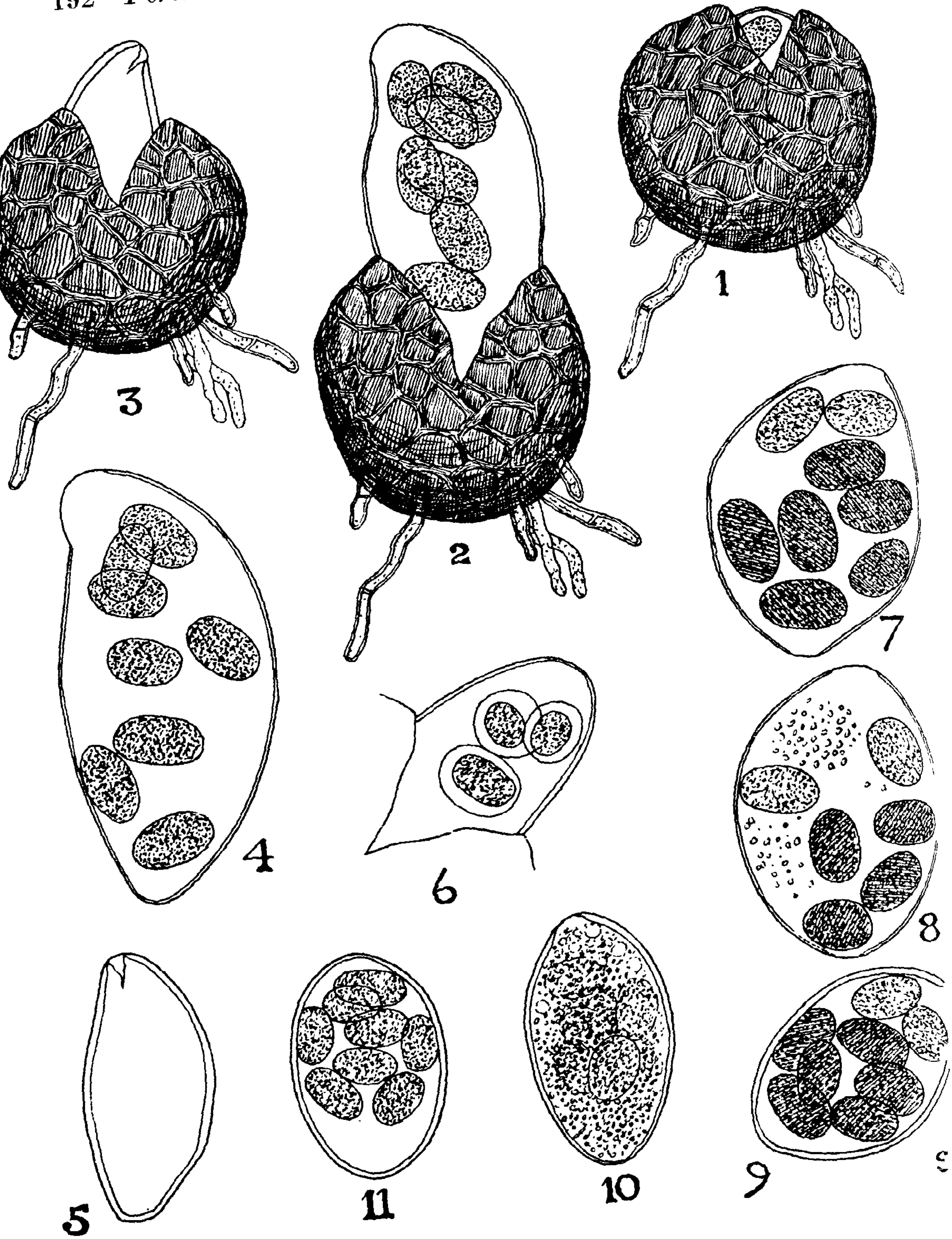
several times, both with asci partly included in the perithecium and when free in the water.

In all cases observed the ripe ascus, i.e. one capable of swelling up in water and discharging the spores, is (with the exception of the granular ascospores) hyaline (see Figs. 2, 4). During the process of the absorption of water, clear vacuole-like spaces arise round the ascospores (Fig. 6). These clear spaces vary in shape and size, and disappear and reappear when the ascus is made to shrink or swell.

A somewhat remarkable phenomenon has been observed under the following circumstances. If a living ripe ascus with hyaline protoplasm is given a certain amount of pressure—not sufficient to rupture the wall—a change is induced in the appearance of the protoplasm (epiplasm), which now becomes opaque and densely granular (and often somewhat vacuolate or "frothy") so that the outline of the ascospores is nearly lost. (Cf. Figs. 10 and 11.) If the ascus at the time of treatment has swollen up in water, the pressure must apparently expel some fluid from the ascus, since its volume after the treatment is much smaller. The ascus is now, so far as I have observed, rendered incapable of swelling to the normal proportions and discharging its spores. If a solution of salt is now added, some amount of further shrinking takes place; the granular substances are dissolved very rapidly, and the ascospores are again visible, surrounded by the protoplasm as hyaline or almost as hyaline as before. If now distilled water is added, the hyaline protoplasm again becomes densely granular, this change taking place almost instantaneously. The process can be repeated many times. Experiments are now being made to ascertain the nature of these changes.

It may be mentioned here that all the examples obtained up to the present of the winter-stage which has been exposed on the bush through the winter have not shown, in February, any mature asci in the perithecia. Investigations are being made to ascertain under what conditions, if any, such perithecia mature, or whether it is only those perithecia which become mature *before* the advent of winter that cause the infections in the following spring.

During 1913 the mildew was found beginning to develop the winter-stage as early as May 26; by June 6 the winter-stage was found in abundance well-developed on the berries of a number of varieties of gooseberries. The winter-stage may be formed almost at the beginning of an attack; in one case (observed in a commercial plantation in Kent) the process of inoculation, incubation and the development of both the summer- and winter-stages occupied only 11 days.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EXPLANATION OF FIGURES.

FIGS. 1—3. A perithecium of *S. mors-uvae* (in water), showing the manner of the dehiscence and discharge of the ascospores. Fig. 1. The wall splits at the apex of the perithecium, exposing to view the apex of the ascus, which immediately begins to swell up and protrude. Fig. 2. The stage reached in about 5 minutes; the ascus has now swollen to about 6 times its original volume, and in so doing has enlarged the opening in the wall of the perithecium; the wall of the ascus in consequence of the enlargement has become very thin, and is in a state of great tension. The ascospores are clearly visible in the hyaline contents of the ascus. Clear vacuole-like spaces (not shown here) are formed round the spores during the process of the swelling of the ascus (see Fig. 6). When the ascus has swollen to its full extent, the wall, after a little time, splits by a slit at the apex, at a place (or "pore") where from the first the wall is thinner (cf. Fig. 9), and the ascospores, all together, are forcibly expelled. The empty and shrunken ascus, now showing again the thick wall, retreats partly into the perithecium, the walls of which draw together somewhat at the opening.

FIG. 4. A fully swollen ascus, which has slipped out of the perithecium into the surrounding water.

FIG. 5. The same ascus as in Fig. 4, after it had discharged its ascospores.

FIG. 6. A ripe ascus beginning to swell up; clear vacuole-like spaces are formed round the ascospores.

FIG. 7. A ripe ascus swollen up in water; on treatment with the neutral red stain, 6 of the ascospores (shown shaded) became deeply stained, while 2 remained unstained.

FIG. 8. The same ascus, on treatment with distilled water; 2 of the stained ascospores burst.

FIG. 9. The same ascus, contracted on treatment with a solution of common salt.

FIG. 10. An ascus, which was previously of the size and appearance shown in Fig. 2, contracted as the result of pressure (see p.191). The contents (excluding the ascospores), which were before hyaline, are now densely granular, so that the outlines of the spores are almost hidden.

FIG. 11. The same ascus as in Fig. 10; after treatment with a solution of common salt, which causes the granular substances to dissolve.

THE PREPARATION AND COMPOSITION OF LIME-SULPHUR SPRAYS.

By A. A. RAMSAY.

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In the preparation of lime-sulphur solutions used for spraying purposes by boiling together lime, sulphur and water, the following equations represent reactions which might occur primarily:

$$(1)\quad 3CaH_2O_2 + S_4 = 2CaS + CaS_2O_3 + 3H_2O,$$

$$(2)\quad 3CaH_2O_2 + S_{12} = 2CaS_5 + CaS_2O_3 + 3H_2O.$$

According to the above equations 3 molecules slaked lime CaH_2O_2 or 3 molecules of quick lime CaO may combine with 4 to 12 atoms sulphur; or 1 part of quick lime (by weight) should combine with ·7619 to 2·2857 parts of sulphur.

The question of how much water should be present for the reaction naturally arises. On this point various suggestions have been made of which the following are examples:

Authority	Formula			Equivalent to		
	Lime lbs.	Sulphur lbs.	Water galls.	Lime lbs.	Sulphur lbs.	Water lbs.
1. *Farmers and Fruit Growers' Guide*, Dep. Agric. N.S.W.	22·7	20	60	1	0·88	26·4
2. Michigan Agric. College Experiment Station	60	125	60	1	2·08	10·0
3. Illinois Formula	15	15	50	1	1·00	33·3
4. Missouri State Board of Horticulture	8	8	50	1	1·00	62·5
5. "Better Fruit," U.S.A. Dep. Agric.	50	100	50	1	2·00	10·0
6. Wagga Formula, N.S. Wales	4½	6	50	1	1·33	111·1

1. *Farmers and Fruit Growers' Guide*, 5th ed. Government Printer, N.S. Wales, 1904, p. 378.
2. *Technical Bulletin*, No. 6, Michigan Agric. College Experiment Station, 1911.
3. *Agric. Gazette*, N.S. Wales, XXI. p. 643.
4. From *Bulletin* 2, Missouri State Board of Horticulture, by W. M. Scott, Pathologist, U.S.A. Dep. Agric.; *Agricultural Gazette*, N.S. Wales, XXII. p. 917.
5. Quaintance and Scott, "Better Fruit," U.S.A. Dep. Agriculture; *Agric. Gazette*, N.S.W., XXIII. p. 990.
6. As used at the Government Fruit Farm, Wagga, N.S. Wales.

From the foregoing table it is noted, that 1 part of lime is associated with ·88 to 2·08 parts of sulphur and 10 to 111 parts of water by weight.

If it be possible to average such wide limits, we would get a mean of 1 part lime, 1·38 parts sulphur, 42·2 parts water by weight.

The following experimental work has been carried out with a view of ascertaining the best proportions of lime and sulphur to be used.

Experiment 1. 25 grams lime CaO + 19·0475 grams sulphur + 250 c.c. water were boiled together under a reflux condenser. The mixture started to boil in 3 minutes: frothed badly and settled into a steady boil in 30 minutes. Boiling continued $1\frac{1}{4}$ hours. The whole was diluted largely, filtered and made up to 500 c.c.

Methods of analyses.

Dhuique-Mayer's method[1], Podreschetnikoff's[2] method, and that of Dusserre and Vuilleumier[3], were tried and abandoned as unsatisfactory. The methods described by Jas. E. Harris[4] were adopted with slight modification and gave excellent results.

In using sodium peroxide as an oxidising agent to convert sulphides of alkaline earths into sulphates, the method as recommended by Harris, which is apparently Modrakowski's method[5], is as follows: "10 c.c. of diluted solution is placed in a tall beaker, covered with a watch glass and 5 or 6 grams sodium peroxide added. After standing a few minutes hydrochloric acid is added, stirring until the solution clears up...after boiling a few minutes to drive off dissolved gases the sulphur may be precipitated as barium sulphate."

I have followed this method and have failed to obtain concordant duplicates.

The reason, I find, is due to the presence of higher oxidised products—chlorates, for such a solution discharges the colour from methyl orange, and from indigo. This fact has been noted by Pringsheim[6].

[1] F. Dhuique-Mayer, *Rev. génér. Chim. pure appl.*, 1908, XI. pp. 273, 274; *Analyst*, 1908, p. 484.

[2] E. Podreschetnikoff, *Zeit. Farben Ind.*, 1907, VI. p. 388; *Analyst*, 1908, p. 141.

[3] C. Dusserre and V. Vuilleumier, *Chem. Zeit.*, 1909, XXXIII. p. 1129; *Analyst*, 1909, p. 545.

[4] Jas. E. Harris, *Technical Bulletin*, No. 6, Michigan Agric. College, 1911.

[5] G. Modrakowski, *Zeit. Physiol. Chem.* 1903, XXXVIII. p. 562; *Analyst*, XXVIII. p. 321.

[6] H. H. Pringsheim, *Berichte*, 1903, XXXII. 4244–4246; *Analyst*, XXIX. p. 97.

These higher oxidised products may be removed, and concordant results obtained, if the solution be reduced with a little potassium iodide, and the excess of iodine removed by boiling. With this alteration I find the method gives excellent results.

The 500 c.c. resulting from Experiment 1 had a specific gravity of 1·0462, and had the 500 c.c. been concentrated to 250 c.c. the calculated sp. gr. would be 1·0924, equal to 12·8 degrees Baumé. The 500 c.c. gave upon analysis the following figures in grams per 100 c.c.:

Monosulphide sulphur	Polysulphide sulphur	Thiosulphate sulphur	Sulphate and sulphite sulphur	Total sulphur	Lime	Lime * calculated
0·58	2·02	1·13	0·07	3·80	2·00	2·12
The various forms in which the sulphur occurs expressed in percentage of total sulphur are						
15·26	53·16	29·74	1·84	100·00	—	—

* CaO calculated as equivalent to Ca necessary to combine with Monosulphide S + Thiosulphate S + Sulphate and sulphite S.

The 500 c.c. solution contains 19·00 grams sulphur and 10·00 grams lime (CaO), while we had at the commencement 19·04 grams sulphur and 25·00 grams lime.

Therefore there remains undissolved 15·00 grams lime (CaO).

Experiment 2. 25 grams lime, 57·1425 grams sulphur, 250 c.c. water boiled together under reflux condenser. Almost all was dissolved in 30 minutes; boiling was continued for 1¼ hours. The fluid was cooled, largely diluted, filtered, and made up to 500 c.c.

The resulting liquid had a sp. gr. of 1·1144. Had this been concentrated to 250 c.c., the sp. gr. (calculated) would have been 1·2288, equal to 28·28 degrees Baumé.

Upon analysis the following figures were obtained in grams per 100 c.c.:

Monosulphide sulphur	Polysulphide sulphur	Thiosulphate sulphur	Sulphate and sulphite sulphur	Total sulphur	Lime	Lime calculated
1·50	6·92	2·54	0·16	11·12	5·00	5·13
The various forms in which the sulphur occurs expressed in percentage of total sulphur are						
13·49	62·23	22·84	1·44	100·00	—	—

The 500 c.c. of solution therefore contains 55·60 grams sulphur, 25·00 grams lime (CaO), while we had at commencement 57·14 grams sulphur, 25·00 grams lime (CaO).

Therefore we have not used 1·54 grams sulphur.

These experiments show that in the proportion of 25 lime and 38·1 sulphur (that is $3CaO$ to S_4), we have too much lime, and in the proportion of 25 lime and 57·14 sulphur (that is $3CaO$ to S_{12}) we have too much sulphur.

The correct proportions when neither lime nor sulphur will be left in excess will therefore lie between these limits.

It is seen that in Experiment 1, 10 grams lime united with 19 grams sulphur, that is 1 part lime to 1·9 parts sulphur, and in Experiment 2, 25 grams lime united with 55·6 grams sulphur, that is 1 part lime to 2·22 parts sulphur. The correct proportion therefore is indicated as lying between 1·9 and 2·2 parts sulphur for every 1 part of lime.

The following four experiments were performed to afford more definite information on this point:

Experiment	Ratio lime to sulphur	Lime lbs.	Sulphur lbs.	Water gallons	Lime grams	Sulphur grams	Water cubic centim.
A	1 to 2·2	50	110	50	25	55	250
B	1 to 2·1	50	105	50	25	52·5	250
C	1 to 2·0	50	100	50	25	50	250
D	1 to 1·9	50	95	50	25	47·5	250

Experiment A. 25 grams lime, 55 grams sulphur, 250 c.c. water boiled together 1¼ hours under reflux condenser. At the end of this time some undissolved matters were left.

The resulting fluid was cooled, and decanted off. The sp. gr. was 1·1822 = 23·4 degrees Baumé.

The fluid on analysis gave the following figures in grams per 100 c.c.:

Mono-sulphide sulphur	Poly-sulphide sulphur	Thio-sulphate sulphur	Sulphate and sulphite sulphur	Total sulphur	Lime	Lime calculated
2·72	10·09	3·55	0·33	16·69	8·36	8·45
The various forms in which the sulphur occurs expressed in percentage of total sulphur are						
16·30	60·46	21·27	1·97	100·00	—	—

In the 250 c.c. solution therefore we have 20·9 grams lime and 41·73 grams sulphur, or 83·6 °/₀ of the lime, 75·87 °/₀ of the sulphur. We had at commencement 25·0 grams lime and 55 grams sulphur.

Therefore we did not use 4·1 grams lime and 13·27 grams sulphur, or 16·4 °/₀ of the lime and 24·13 °/₀ of the sulphur.

Experiment B. 25 grams lime, 52·5 sulphur, 250 c.c. water boiled together under reflux condenser for $1\frac{1}{4}$ hours.

The fluid portion was decanted from the sediment and had a specific gravity of 1·1935 equal to 24·6 degrees Baumé.

The fluid on analysis gave the following figures in grams per 100 c.c.:

Mono-sulphide sulphur	Poly-sulphide sulphur	Thio-sulphate sulphur	Sulphate and sulphite sulphur	Total sulphur	Lime	Lime calculated
2·78	11·38	3·93	0·31	18·40	8·59	8·84
The various forms in which the sulphur occurs expressed in percentage of total sulphur are						
15·11	61·85	21·36	1·68	100·00	—	—

We started with 25 grams lime, 52·5 grams sulphur. Found in 250 c.c. fluid 21·48 grams lime, 46·00 grams sulphur, or 85·92 °/₀ of the lime, 87·62 °/₀ of the sulphur.

Therefore balance 3·52 grams lime, 6·50 grams sulphur = 14·08 °/₀ of the lime, 12·38 °/₀ of the sulphur.

Experiment C. 25 grams lime, 50 grams sulphur, 250 c.c. water treated as in A and B.

The resulting fluid had a sp. gr. of 1·200 = 25·3 degrees Baumé, and gave on analysis the following figures in grams per 100 c.c.:

Mono-sulphide sulphur	Poly-sulphide sulphur	Thio-sulphate sulphur	Sulphate and sulphite sulphur	Total sulphur	Lime	Lime calculated
3·09	10·54	3·92	0·31	17·86	9·31	9·37
The various forms in which the sulphur occurs expressed in percentage of total sulphur are						
17·30	59·01	21·95	1·74	100·00	—	—

We started with 25·0 grams lime, 50 grams sulphur. Found in 250 c.c. fluid 23·28 grams lime, 44·30 grams sulphur, equal to 93·12 % of the lime, 88·60 % of the sulphur.

Therefore balance 1·72 grams lime, 5·70 grams sulphur = 6·88 % of the lime, 11·40 % of the sulphur.

Experiment D. 25 grams lime, 47·5 grams sulphur, 250 c.c. water treated as before described.

The resulting fluid had a sp. gr. of 1·1921, equal to 24·4 degrees Baumé.

The fluid on analysis gave the following figures in grams per 100 c.c.:

Mono-sulphide sulphur	Poly-sulphide sulphur	Thio-sulphate sulphur	Sulphate and sulphite sulphur	Total sulphur	Lime	Lime calculated
3·09	10·20	3·46	0·31	17·06	9·19	8·97
The various forms in which the sulphur occurs expressed in percentage of total sulphur are						
18·11	59·79	20·28	1·82	100·00	—	—

We started with 25·0 grams lime, 47·5 grams sulphur. Found in 250 c.c. fluid 22·98 grams lime, 42·65 grams sulphur, equal to 91·92 % of the lime, 89·79 % of the sulphur.

Therefore balance 2·02 grams lime, 4·85 grams sulphur, equal to 8·08 % of the lime, 10·21 % of the sulphur.

The results of the experiments A, B, C, and D are summarised in separate table.

These experiments indicate that the most suitable proportions lie between C and D, for in these the highest proportions of lime and of sulphur used have been obtained in the boiled fluid.

In C the ratio of lime to sulphur is 1 to 2·0 and in D 1 to 1·9. It appears, therefore, that the most satisfactory proportions of lime and sulphur is when they are in the ratio of 1 to 1·95.

The question of the most satisfactory quantity of water to be used has not been dealt with exhaustively, but it is hoped that when time permits this will be investigated.

I find that when 25 grams lime, 55 grams sulphur, 250 c.c. water are boiled together the portion which is not in solution, or which we may term residue, amounts to 24·13 % of the sulphur and 16·4 % of the

lime, or about $20\frac{1}{4}$ °/₀ of the total ingredients used, whereas when the above quantities are diluted, filtered, and made up to 500 c.c., only 2·3 grams of residue is left, equal to 2·9 °/₀ of the ingredients used.

Also I find that when 25 grams lime, 52·5 grams sulphur, and 250 c.c. water are boiled together, 12·38 °/₀ of the sulphur used and 14·08 °/₀ of the lime used are not in solution, or about $13\frac{1}{4}$ °/₀ of the ingredients used, whereas when the same quantities are diluted, filtered, and made up to 500 c.c., only 1·95 grams residue is left, equal to $2\frac{1}{2}$ °/₀ of the ingredients used.

Apparently, then, more economy would be effected by diluting the formula 50-100-50 gallons to 50-100-100 gallons.

When lime-sulphur solution is prepared by boiling lime and sulphur together in the proportions of 25 grams lime, $47\frac{1}{2}$ to 55 grams sulphur, in 250 c.c. water it appears the 250 c.c. solution will contain :

Calcium monosulphide 16·4 grams			
containing	9·11 gm. Ca	and	7·29 gm. S
Sulphur ... ... 26·4 grams			
containing			26·40 „
Calcium thiosulphate 22·06 grams			
containing	5·80 „	„	9·29 „
Calcium sulphate ... 3·4 grams			
containing	1·00 „	„	0·80 „
Total calcium ...	15·91		43·78 total sulphur.

The solubility of calcium monosulphide is given as about 2 grams per litre by all authorities I can find, whereas in this mixture we would have 65 grams per litre in solution.

It appears to me that the salt actually present might be the sulphydrate CaH_2S_2 and hydrate $CaOH_2O$ or else the hydroxy-hydrosulphide CaHSOH.

For

$$2CaS + 2H_2O = CaH_2S_2 + CaH_2O_2$$

and $$CaH_2S_2 + CaH_2O_2 \rightleftarrows 2CaHSOH \text{ (reversible action).}$$

The ratio of calcium to combine with the sulphur found as monosulphide sulphur would still be Ca : S.

Again, when manganous sulphate is added to the lime-sulphur solution, manganous sulphide is produced, and a very minute quantity of sulphuretted hydrogen, but when hydrochloric acid is added large

quantities of sulphuretted hydrogen are given off. If the hydroxy-hydrosulphide be the salt present we would have

$$CaHSOH + MnSO_4 = CaSO_4 + MnS + H_2O$$

and

$$CaHSOH + 2HCl = CaCl_2 + H_2S + H_2O,$$

whereas if the hydrosulphide be the salt present we should have

$$CaH_2S_2 + MnSO_4 = CaSO_4 + MnS + H_2S.$$

The monosulphide sulphur therefore appears to be essentially calcium hydroxyhydrosulphide CaHSOH with very minute quantities of calcium hydrosulphide CaH_2S_2.

The solution of lime-sulphur then appears to consist of calcium hydroxyhydrosulphide, calcium thiosulphate, calcium sulphate, with sulphur held in solution.

Summary of Experiments A, B, C, D.

Experiment	Ingredients used, grams		Water used	Ratio		Analysis of fluid per 100 c.c.						Sulphur found expressed in percentage weight of total sulphur					Ingredients in 250 c.c. of mixture, grams		Percentage of ingredients			
																			Used		Not used	
	CaO	Sulphur	c.c.	CaO	Sulphur	Monosulphide sulphur	Polysulphide sulphur	Thiosulphate sulphur	Sulphate and sulphite sulphur	Total sulphur	Lime	Monosulphide sulphur	Polysulphide sulphur	Thiosulphate sulphur	Sulphate and sulphite sulphur	Total sulphur	CaO	Sulphur	CaO	Sulphur	CaO	Sulphur
A	25	50	250	1	2·2	2·72	10·09	3·55	0·33	16·69	8·36	16·30	60·46	21·27	1·97	100·00	20·9	41·73	83·6	75·87	16·4	24·13
B	25	52·5	250	1	2·1	2·78	11·38	3·93	0·31	18·40	8·59	15·11	61·85	21·36	1·68	100·00	21·48	46·00	85·92	87·62	14·08	12·38
C	25	50	250	1	2·0	3·09	10·54	3·92	0·31	17·86	9·31	17·30	59·01	21·95	1·74	100·00	23·28	44·30	93·12	88·60	6·88	11·40
D	25	47·5	250	1	1·9	3·09	10·20	3·46	0·31	17·06	9·19	18·11	59·79	20·28	1·82	100·00	22·98	42·65	91·92	89·79	8·08	10·21

A BACTERIAL ROT OF CELERY.

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During the month of February, 1913, an examination was made of a number of celery plants, grown in the gardens attached to the South-Eastern Agricultural College, Wye, for the purpose of ascertaining the amount of damage produced at that time of the year by the parasitic fungus *Septoria Petroselini* var. *Apii*. Some of these plants were found to be in an advanced stage of decay, the affected portions being discoloured and pulpy, and showing every evidence of a soft, brown rot. As numerous pycnidia of the fungus were to be found on most of the plants examined and as that parasite is known to continue its ravages during the winter, even when the plants are dug and placed in storage[1], it was thought that the Septoria was the primary cause of the damage. It was found, however, in most cases, that when the soft pulp was subjected to microscopic examination, there was no trace of either pycnidia or mycelium, while bacteria and often eelworms were swarming in such material. Again, when the tissues on the border line of the sound and the decaying parts were examined it was exceptional to find any extraneous organisms with the exception of actively motile bacteria which were found within the cells and in the liquid oozing from the decaying mass. These facts suggested that the bacteria were directly responsible for the decay of the celery. In order to test this it was decided to isolate the organism and attempt to reproduce the symptoms of the disease by inoculations from pure cultures.

[1] Duggar in *Fungous Diseases of Plants*, p. 361, says "The late blight is destructive in the field until the plants are 'lifted.' It may also extend its injuries to the storage coop or cellar."

In some instances outer leaves only were attacked, and when these were removed the plants appeared little or no worse, but in others the stem ("heart") itself was undergoing decay (see Fig. 1, Plate II), when the damage was of a more serious nature. At that time of the year it is difficult, if not impossible, to find celery plants free from injury of some kind, and particularly are they subject to the attacks of snails and slugs which, in all probability, not only disseminate pathogenic organisms but also introduce them into the internal tissues of the plant. Many petioles showed a wrinkling of the inner (ventral) surface, due to unequal growth of the inner and the outer tissues; this condition, often found in, but not restricted to, diseased plants, leads to a rupture of the internal cells, thus favouring the development of those rot-producing bacteria which gain an entrance. Fig. 6, Plate II shows such a petiole. Above are seen two, more or less circular holes, similar to those caused by slugs; such holes are usually on the inner side, but sometimes on the outer, and, extending inwards to about midway between the two surfaces, expose to bacterial attack the more delicate internal cells which are normally protected by the vascular strands, the collenchyma and the epidermis. Beneath the wrinkles of this particular specimen was a space, covered on the side towards the axis by a layer of tissue about 1 mm. thick, while on the other side the thickness was about 3 mm. The tissue lining the space was a light yellowish brown; two strips, a little paler in colour and with white, healthy flesh between them, connected the space with the holes above mentioned. On placing a little of the brown tissue in water swarms of motile bacteria floated out together with a few eelworm (Rhabdites) larvae. From the bacteria so obtained was prepared a series of three isolation, poured-plate cultures[1] in celery-extract agar[2]. After incubation for 24 hours at 26° C. Nos. 1 and 2 of the series showed numerous colonies, but in No. 3 isolated colonies were obtained and from one of these another series of plates was made; finally tube cultures were prepared from the resulting isolated colonies and a form was obtained which in poured plates of celery-extract agar produced three forms of colonies similar to those shown in Figs. 8 and 9, Plate II, and described below on p. 212. The organism isolated was found to liquefy gelatin prepared with celery-extract.

On March 11th a celery plant with well-developed "heart-rot" (similar to the one shown in Fig. 1) was found to be attacked by

[1] The photograph was taken on Feb. 26th and the cultures were started the same day.

[2] For the preparation of this medium see footnote on p. 212.

motile bacteria. From this material a form was isolated, apparently similar to the one previously isolated; sub-cultures were made on various media, and inoculations as shown below (pp. 207–211) were made proving its pathogenicity. Details of its behaviour in those media and the results obtained from the inoculations are given.

PREVIOUS WORK ON BACTERIOSIS OF CELERY.

Although much work has been done in recent years on bacteriosis of vegetable tissues, with the result that a considerable number of forms pathogenic to plants have been isolated, a perusal of works on the subject showed few references to bacterial rots of celery. General works on plant diseases (including bacteriosis) make no mention of celery in this connection, so far as could be ascertained, with the exception of Sorauer's *Handbuch* [(13)], in which on p. 61 reference is made to two investigators who had observed a celery-rot resembling in some respects the one here described.

A disease of celery leaves attributed to bacteria was first noticed by Halsted [(5)], who in 1892 announced its appearance in America in a *Bulletin* of the New Jersey Agricultural Experiment Station. I have not seen Halsted's original paper but Mr M. T. Cook, the Plant Pathologist at that Station, informs[1] me that the bulletin referred to "does not contain a description of the organism causing bacterial rot but merely a description of the disease." He also states that the rot has recently been again reported at that Station.

A celery-rot appeared in Italy in 1896 and was described by Brizi [(1)], who stated that the disease was at that time new to Italy[2]. This observer isolated a motile bacillus from the material and cultivated it on a few prepared media; it grew well on nutrient agar and nutrient gelatin, but proved unable to liquefy the latter. The form thus obtained he named *Bacterium Apii*[3].

That the disease is still troublesome in Italy is affirmed by Prof. Saccardo who, writing to me in January of the present year, says "Je veux vous informer, moi-même, que aussi chez nous, particulièrement en province de Treviso cette bacteriose fait beaucoup de ravage!"

[1] Letter dated Dec. 15th, 1913.

[2] "La malattia bacteriologica ora descritta...ha menata una vera strage nella coltivazioni dei sedani, e tale malattia, se non è del tutto nuova, certa non era stata mai finora osservata nè descritta in Italia."

[3] =*Bacillus Apii* (Brizi) Migula.

A paper by Klebahn (9) on "Krankheiten des Selleries," in 1910, includes a short discussion on the observations made by Halsted and Brizi; reference is also made to an experiment by Ritzema-Bos (10), who isolated a bacterium and reproduced the disease but gave no details of the organism or methods.

The only reference I can find to a celery-rot recorded from the British Isles is in a paper by Johnson and Adams (6), who merely state however "that a brownish rot, similar to that of cabbage, has occurred in celery from Counties Derry and Tyrone and is caused by a motile bacterium occurring singly or in rod-like colonies and measuring $1{\cdot}4 \times 0{\cdot}7\mu$." Prof. Johnson, to whom I applied for further information, wrote in reply[1] that no further work was done on that subject by himself and his colleague.

It would appear that the only organism isolated from celery and adequately described is the form obtained by Brizi. Though that form is not mentioned as attacking the "heart," as is the case with the one described in the present paper, it is possible that under suitable conditions it would do so. It seems certain, however, that the two are not identical, for Brizi's bacillus was incapable of liquefying gelatin. Thus he states[2]: "Sulla gelatina di brodo di vitello peptonizzata col, 10 °/₀ di gelatina, cresce bene ad una temperatura di 20—22°; le colonie...crescono in superficie senza affondare nel substrato, e per conseguenza senza fondere la gelatina." This point he still further emphasizes[3]: "Le colonie, lasciate a sè medesime, dopo un certo tempo si intorbidano si fanno gialliccie ma senza fondere mai in nessun caso la gelatina." On the other hand the form isolated at Wye readily liquefies gelatin, prepared either with peptonized bouillon or with an extract of celery, when grown in stab, streak, or plate cultures. More than twenty such cultures, started at various times, ranging over a period extending from March 1913 to Jan. 1914, have in every case produced liquefaction of the gelatin. In peptonized bouillon itself the liquefaction is particularly rapid (see below, p. 214 and Figs. 10 and 11).

Taking these facts into consideration it is proposed to designate the organism, the cultural characters of which are given towards the end of the paper, as *Bacillus apiovorus*, nov. sp.

[1] Letter dated Nov. 5th, 1913.

[2] *Cent. f. Bakt.*, Abt. II. Vol. 3, p. 579.

[3] *Idem.*

INOCULATION EXPERIMENTS WITH *BACILLUS APIOVORUS*.

Experiment 1.

Preliminary experiment Mar. 12th, 1913.

A portion of celery "heart" was washed under the tap, slices cut with a flamed scalpel and dropped into distilled water: the slices were cut into sectors and five of these were placed in each of two sterile petri dishes:

Dish (1). Three portions were inoculated from the diseased celery heart mentioned on p. 204; two pieces control.

Dish (2). As in (1) but inoculated from colonies obtained in an isolation poured-plate culture prepared from the same celery material.

On March 14th the three inoculated pieces in both (1) and (2) showed discoloration indicating the commencement of the rot; one in dish (1) was brown over the whole surface. All four controls showed no discoloration. Particles of the inoculated pieces in (2) were examined microscopically and found to be swarming with motile bacteria.

Experiment 2.

A portion of heart after washing under the tap was soaked in 0·1 °/₀ solution of mercuric chloride for ½ min., then washed with sterilized water: slices were cut with a sterile razor, dropped into sterile petri dishes and cut into four pieces with a flamed scalpel.

Inoculations made March 18th.	*Result April 5th.*
A. Dish (1). Two pieces inoculated from a colony of a poured-plate culture, two kept as controls.	(1) One inoculated piece showed a discoloured patch 0·5 cm. in diameter; the other was discoloured over half its surface: controls unchanged.
Dish (2). As in (1).	(2) Each inoculated piece showed a discoloured patch 0·5 cm. in diameter; controls unchanged.
Dish (3). Not inoculated—all four pieces kept as controls.	(3) No change.

B. All the outer leaves were removed from a celery plant, leaving a portion of stem several inches long bearing young leaves; the whole was soaked in the corrosive sublimate solution for ½ min., washed with sterilized water and the upper portion bearing young leaves was cut away with a sterile razor. The remainder was placed in a beaker on

a petri dish cover and the whole covered by a large beaker (all the glassware used, previously sterilized) after inoculating the cut surface.

On April 5th a dark brown discoloration was seen extending from 1·8 cm. to 3·3 cm. from the upper cut surface where the inoculation had been made. On cutting longitudinally the discoloration and soft rot were found to extend to 4·6 cm. from the inoculated surface. This specimen is shown in Figs. 3 and 4.

Experiment 3.

April 7th. Two slices of celery heart were obtained as in Expt. 2, except that the stem from which they were cut was soaked in the corrosive sublimate solution for 1 min. Each slice was placed in a sterile petri dish and cut into halves, of which one was inoculated the other kept as control.

Result: On April 11th, after having been kept at a temperature of 8°—10° C. for four days, the inoculated pieces were almost completely brown and soft, a little of the tissue towards the periphery being still firm. One of these slices is shown in Fig. 5.

Experiment 4.

On May 17th an attempt was made to infect seedling celery plants having leaves 3 to 4 ins. in length. In each of three pots two plants were treated as follows:

Plant No. 1. (*a*) One petiole cut across just below the insertion of the lamina and the inoculation made on the cut end.

(*b*) Bacteria from a colony of a poured plate were placed on the petiole just below the lamina and 3 pricks were made with a sterilized needle through the bacterial mass.

Plant No. 2. (*a*) One petiole treated as in 1 (*a*) but not inoculated.

(*b*) One petiole treated as in 1 (*b*) but not inoculated.

Result: On May 20th plant No. 1, inoculation (*a*), showed discoloration for 0·2—0·4 cm. below the inoculated surface. No. 2 (*a*) in each of the three pots showed no change. Later the three petioles thus inoculated on the cut surface became rotten to the base and one of the controls also became infected.

Those inoculated by needle-pricks gave no positive result except a slight distortion of the petiole in two of them.

All the plants grew on and the rot did not pass from the infected petioles 1 (*a*) to the rest of the leaves.

On June 11th similar inoculations, made by pricking as in 1 (*b*) above, were performed on young plants growing in pots, but again with negative results. As will be seen below (see Expts. 7, 8 and 9) older plants grown in pots were readily infected[1] by needle-pricks through a bacterial emulsion placed on the leaf-stalk. (Experiments with young seedlings will be repeated this year to test their apparent partial resistance to the bacteriosis.)

Experiment 5.

Through pressure of other work the inoculation experiments were discontinued during the summer months and not resumed until November; meanwhile the organism was grown in tube cultures, chiefly on celery-extract agar[2].

On Nov. 8th a celery plant was removed from the ground and after washing with ordinary tap-water, then with sterilized tap-water, six of the leaves were cut across about 1 inch above the lowest pair of leaflets and inoculations made on the cut ends of three of them. After two days the latter showed the typical browning indicative of the rot, but the progress of the rot itself was very slow; in one case only had it extended 1 cm. from the inoculated surface by Nov. 17th. The slow development of the organism at this time was probably due to its continued growth as a saprophyte, for that particular strain used in this experiment had been growing on prepared media only, from April 5th onwards.

Loss of virulence is known to occur in pathogenic bacteria in cultures; thus Smith [(11)] in Vol. II of his monograph writes[3]: "In some species long cultivation on artificial media destroys or generally weakens the ability of the organism to attack tissues," and a brief treatment of the subject is to be found in the same volume[4]. *Bacillus tumaefaciens,* the crown-gall organism, was found by Smith, Brown and Townsend [(12)] to lose its virulence in cultures.

[1] The plants used in the later experiments were from the same batch as those used in Expt. 4 (*i.e.* same variety, "Sandringham White") and had been growing in pots throughout the summer.

[2] This medium is easily prepared (see footnote on p. 212), and sets well after either discontinuous sterilization or autoclaving.

[3] *loc. cit.*, p. 65.

[4] *loc. cit.*, p. 94.

Experiment 6.

From the petiole treated in Expt. 5 the organism was re-isolated on Nov. 17th, and on the following day plants, removed from the soil and placed with their roots in beakers with a little water, were inoculated.

Inoculations Nov. 18th.	*Result.*
Plant No. 1. Two petioles were cut across between the second and third pairs of leaflets; one was inoculated on the cut surface, the other kept as control.	On Nov. 20th the rot had extended to 2·5 cm., and on Nov. 26th to 15 cm. from the inoculated surface. The control showed no change.
Plant No 2. From the inner surface of each of two petioles a small portion of tissue was removed (to simulate the hole made by a snail). One was inoculated, the other kept as control.	On Nov. 20th the rot had extended across the whole width of the petiole and the part of the leaf above the inoculated portion fell over. (This stage is shown in Fig. 7, Plate II.) The control was unchanged. By Dec. 4th the rot had reached to the base of the inoculated leaf and was attacking the other leaves from below.

Experiment 7.

On Nov. 20th bacteria were procured from the inoculated petiole of Plant 2 in the last experiment and the organism was re-isolated; isolated colonies were obtained by means of plate cultures, from one of which a tube culture was prepared on Nov. 23rd. The latter was used in the following experiment.

Inoculations made Nov. 24th.	*Result.*
Two leaves of a plant growing in a pot were inoculated by placing bacteria midway between the two lower pairs of leaflets and making four pricks with a sterilized needle through the bacterial mass.	On Nov. 26th the two leaves fell over above the point of inoculation; in one case the rot had extended for 2·5 cm. above the point of inoculation and the same distance below, in the other for 3 cm. both above and below.
Two other leaves were similarly pricked without previous application of the bacteria.	The controls showed no change.

Experiment 8.

With a culture of the same strain as that used in Expt. 7, other inoculations were made on a plant growing in a pot:

Inoculations made Jan. 17th, 1914.	*Result.*
(1) Two petioles were inoculated 1 cm. below the lowest pair of leaflets and one prick only made in each case through the bacterial emulsion. Two petioles were similarly pricked but bacteria not applied.	One was found on the morning of Jan. 20th to have fallen over from the point of inoculation during the night; the petiole was discoloured for 1 cm. above and 3 cm. below the point of inoculation. The other collapsed during the afternoon of the 20th, when the rot had extended 1 cm. upwards and 2 cm. downwards. The controls were unchanged.
(2) A petiole was cut transversely immediately below the lowest leaflets and bacteria were placed on the cut end. Another petiole was treated in the same manner but the cut end was not inoculated.	On Jan. 20th the inoculated petiole was discoloured to 1 cm. below the cut end, and on the 22nd a length of 4·5 cm. was completely rotten and collapsed.

Experiment 9.

On Jan. 20th bacteria were obtained from one of the petioles inoculated in the last experiment, and the organism was again isolated. A tube culture was prepared and from this, on Jan. 24th, two petioles of a plant in pot were inoculated as in Expt. 8, two others acting as controls.

Both inoculated leaves fell over from above the point of infection during the night of the 26th.

Whether celery plants without wounding can be directly infected from mere contact with diseased organs is uncertain. It has been noticed, however, that where diseased leaves have collapsed and fallen across healthy ones, the latter have acquired the rot. This was seen in the plant inoculated on Jan. 17th (Expt. 8). One of the infected petioles as it decayed fell across the laminae of two healthy leaves; of these, when observed on Jan. 30th, one had lost the whole of its lamina with the exception of the two lowest leaflets which were hanging from their decaying petiolules, the other showed that the rot had destroyed one of the lowest leaflets to its base and was advancing on the rest of the leaf, the lamina falling over from this point on the following day.

Celery leaves are so very brittle that it is probable in some of these cases that the weight of the pulpy mass on the leaves has broken the petiolules of the leaflets and allowed entrance to the bacterium. Experiments made by placing bacteria on healthy leaves have not hitherto yielded definite results; in one case a few small discoloured

patches, which failed to increase in size, were found eight days after the leaves were so treated.

The Organism causing Heart-rot of Celery.

The celery heart-rot found at Wye is caused by an actively motile, rod-shaped bacterium, found singly or in pairs joined end to end. When taken from infected plants or from young cultures and mounted in water, the single rods measure $2{\cdot}5$—$3{\cdot}5 \times {\cdot}6$—${\cdot}7\mu$ and the double rods are 5—$6{\cdot}5 \times {\cdot}6 \times {\cdot}7\mu$; occasionally longer rods (up to $16{\cdot}5\mu$ in length) with ill-defined septa are met with and probably represent four single rods. They stain readily with carbol fuchsin and with gentian violet; Loeffler's and Muir's Pitfield methods of staining have shown the presence of two to twelve peritrichiate flagella. No endospores have been observed.

It grows readily on agar prepared with an extract of celery heart or petioles[1], and its behaviour in pure cultures was first studied in this medium. In poured plates incubated at 26° C. colonies of three kinds can readily be distinguished within 24 hours, viz.:

(1) Those on the surface are convex, circular, with an entire margin, usually about 1—2 mm. in diameter, glistening, opalescent, almost white but with a slight yellowish tinge. In old cultures thinly sown, the colonies may become lobed.

(2) Those embedded in the agar are more or less fusiform, measuring usually about $0{\cdot}4 \times 0{\cdot}2$ mm. and appear much more dense and of a deeper colour when viewed by transmitted light than those on the surface.

(3) In addition to the above other colonies are to be found in the plane where the agar comes into contact with the bottom of the plate; these are in general about 1—1·5 mm. in diam., circular, white or almost so, and by reason of their situation do not appear highly refractive as do those on the surface of the agar (see Figs. 8 and 9, Plate II, where the surface colonies appear to have a dark centre and light margins,

[1] This medium is prepared as follows: clean, healthy portions of celery "heart" are selected or petioles may be used; these are cut up into small pieces, added to twice their weight of distilled water in a beaker and steamed gently for two hours. The liquid is poured off, filtered, *allowed to cool*, and filtered again. The resulting liquid (which should be quite free from suspended matter *when cold*) is again heated and an equal volume of hot 3 °/₀ agar solution added. It is then steamed for a short time and filtered hot; it may be sterilized by the discontinuous method or by autoclaving at 115° C. for 20 minutes, though the latter method tends to discolour the extract.

while those in contact with the glass are uniformly white). At times these colonies are larger and have radiating lobes.

To show the relation between these deeply embedded colonies and those at the surface, the plates shown in the photographs were prepared, one (Fig. 8) from a surface colony, the other (Fig. 9) from a deeply embedded circular colony of a plate culture which was similar to those illustrated, except that the colonies were fewer in number enabling sub-cultures to be made without contamination from neighbouring colonies. The colony-forms (1) and (2) are usually not sharply delimited, for transitional forms occur, while there are no such transitions between the forms (2) and (3).

As plate cultures become dry the colonies lose their whitish opalescence and are eventually almost as transparent as the agar itself, although they retain a yellowish-brown tint.

Growth on streak cultures of celery-extract agar is fairly rapid; in 24 hours, whether the tubes are incubated or kept at room temperature, the streak is continuous, about 2 mm. broad at the middle, has a slight tinge of yellow (sebaceous), margin undulate or almost entire.

Peptone-bouillon agar streaks are similar to the last but usually more translucent and margin less undulate.

Growth on agar prepared with prune-juice is very slow and is practically confined to the original line of the streak, remaining therefore very narrow.

Streak cultures in which the water of condensation has flowed over the streak develop an iridescent film after a few days.

As already stated gelatin prepared either with celery-extract or peptone-bouillon is readily liquefied. In the bouillon-gelatin (10 %, gelatin) liquefaction is rapid, and 10 c.c. (as stab-culture) may be completely liquefied within 3 days (see below, p. 214).

When the pathogenicity of the organism was established it was thought desirable to study its cultural characteristics in accordance with the "Numerical System of Recording the Salient Characters of an Organism" elaborated by the Society of American Bacteriologists, described in a bulletin by Harding [3] and used by Erwin F. Smith in his *Bacteria in Relation to Plant Diseases.* With this end in view a series of tube cultures was started on Dec. 5th, 1913, of the strain isolated from the petiole which had become infected after artificial inoculation on Nov. 18th (Expt. 6, Plant No. 2). The cultures were as follows:

Celery-agar streaks, one at room temperature, 14—15° C., the other incubated: Dec. 6th, growth as described above for this medium.

Two *celery-gelatin stabs,* at room temperature: Dec. 6th (24 hrs.) infundibuliform liquefaction in stab, crateriform (2 mm. diam.) at surface, more pronounced on Dec. 8th, by which time it had extended across the whole upper surface: on Dec. 10th still more pronounced, flocculose scum on surface of glass just above the gelatin, dense sediment at apex of stab.

Bouillon-agar streaks, one at room temperature, another incubated: growth as described above for this medium.

Two *bouillon-gelatin stab* cultures (+10 Fuller's scale, gelatin 10°/₀): Dec. 6th (24 hrs.) infundibuliform liquefaction, crateriform above (about 1 cm. diam. at surface); this condition is shown in Fig. 10, the photograph being taken 20 hrs. after inoculation. Dec. 8th (72 hrs.) the whole liquefied, turbid layer extending from surface to 0·5 cm. below, the rest showing a flocculent suspension with sediment at bottom of tube. Dec. 16th turbid layer nearly 2 cm. deep, below this the liquefied gelatin was clear with the exception of a few suspended flocculi, dense whitish sediment (Fig. 11).

Bouillon-gelatin streak: Dec. 6th beaded to undulate, broad, sinking into the gelatin: liquefaction complete on Dec. 8th.

Dextrose Solution with litmus in Durham's tubes, prepared as recommended by Eyre[1] and incubated at 26° C., three tubes inoculated, one control. Dec. 6th (24 hours) solution turbid, red tint well-defined, a little gas; control unchanged.

Lactose Solution, prepared and incubated similarly. Dec. 6th slight turbidity, change of colour not well-defined, intermediate between that of control and the inoculated tubes of dextrose, a little gas; control unchanged: on Dec. 7th (48 hours) red tint distinct in tubes inoculated.

Saccharose Solution, prepared and incubated as above for dextrose. Dec. 6th turbid, distinct red tint, a little gas.

After Dec. 7th, when the inoculated lactose tubes assumed the same tint as those of the other two sugars, the tubes (including controls) showed no appreciable change for over a month, after which the cultures were destroyed. For the behaviour of the organism in fermentation tubes containing these sugar solutions see below.

[1] Eyre's *Bacteriological Technique*, 2nd Ed., 1913, p. 178.

Nitrate Bouillon[1], two tubes inoculated, one control. Dec. 6th (24 hrs.) distinct turbidity in former, latter clear. Dec. 10th the metaphenylene diamine test[2] for nitrites was applied to half the contents of one tube and a deep brownish-red coloration was obtained; the starch and potassium iodide test[3] was applied to the other half and a dark-blue (almost black) colour was obtained. The contents of the control tube were divided, to one portion the former test was applied, to another the latter: no colour reaction resulted in either case. The second inoculated tube was tested a few days later and gave the same reactions as the first (see below for growth in Durham's tubes).

Sterilized Potato (semi-cylinders), one in a Roux tube was incubated, another in ordinary test-tube kept at room temperature. On Dec. 6th the former showed a faint yellow smear, but there was no visible growth on the latter. On Dec. 8th both showed a yellow slimy growth on the surface. On Dec. 19th the liquid at the bottom of the Roux tube was tested with:

(1) Iodine solution, giving a deep bluish-purple coloration.
(2) Fehling's solution, giving no reduction of copper.
(3) Litmus solution, giving neutral reaction.

Starch Jelly, prepared as recommended by Smith[4], two inoculated tubes and one control were incubated. Dec. 6th faint whitish growth along the streak: a slow liquefaction commenced and on Dec. 19th one tube yielded 1·1 c.c. of turbid liquid: this was diluted with a little water and tests applied as for the liquid in the Roux tube with the same result; Jan. 29th 1·2 c.c. was obtained from the second tube and treated in the same way with the same result. The jelly in the control tube had remained solid throughout. Later it was found, in other cultures, that a more distinct purple reaction was given on filtering the turbid liquid, diluting with water and adding iodine solution.

Uschinsky's Solution (with glycerine), (1) tinged with litmus in Durham's tube, (2) in Durham's tube, no litmus, (3) in ordinary tube, no litmus: all three were incubated with a corresponding control of each. Dec. 6th the three inoculated tubes showed a slight turbidity, controls clear. Dec. 15th all three were slightly turbid but with a dense whitish sediment: tube (3) and its control were tested with neutral litmus solution; both gave a slight alkaline reaction, as did also (2) and its control on Dec. 19th. No gas was produced in any case and the controls remained unchanged until the tests were made.

[1] Eyre's *Bacteriological Technique*, p. 185. [2] *Idem.* p. 289.

[3] Smith's *Bacteria in Relation to Plant Diseases*, Vol. I. p. 63. [4] *Idem.* p. 196.

On Dec. 12th two cultures in nitrate bouillon in Durham's tubes were started to determine whether or not gas was produced: growth occurred as before, no gas was liberated and both gave the reaction to the metaphenylene diamine test when applied on Jan. 29th.

On Jan. 23rd a series of cultures similar to the above was started with the strain isolated from an infected petiole artificially inoculated in Expt. 8 (1) and used for inoculations in Expt. 9. Allowing for minor differences, due to manipulation (*e.g.* length of stab or streak), or to slight changes in the consistency of the media[1], the results were identical. Thus bouillon gelatin was liquefied more quickly than celery-extract gelatin; nitrate bouillon gave pronounced nitrite reaction both with the metaphenylene diamine and the starch tests after 5 days, without gas production; growth on sterilized potato was slow, yellow in colour with no corrosion of the surface; the liquid obtained from the starch jelly reacted as before; Uschinsky's solution remained slightly alkaline, the litmus was decolorized, and no gas formed. Even the colour changes in the sugar solutions as observed after 24 and 48 hours were faithfully reproduced, *i.e.* acid reaction appearing more slowly in lactose than in dextrose or saccharose.

Cultures of both strains have also been made in these sugar media in fermentation tubes; the organism grows well in the closed end in every case as shown by a turbidity which can be detected in 24 hours; later this is more pronounced, developing into a finely flocculent suspension and sediment; a little gas is liberated.

The conclusion drawn from these facts is that the two strains, both of which have been shown to reproduce the celery-rot by artificial inoculation, are the same form, the more important cultural features of which are, apart from those already fully treated, as follows: Nitrates are readily reduced to nitrites without the production of gas; starch jelly is slowly liquefied, and the diastatic action is very feeble, though the purplish reaction with iodine denotes some slight change in the starch; vigorous growth occurs with glycerine (Uschinsky's solution) but neither acid nor gas is produced; acid and gas are developed in solutions of dextrose, lactose, and saccharose, and the turbidity observed in the closed end of the fermentation tubes shows that the organism is a facultative anaërobe in those solutions; most of the cultures have a faint yellowish colour, which is most pronounced on sterilized potato.

[1] The second batch of starch jelly was liquefied even more slowly than in the first series and was probably a little firmer.

These characters, arranged according to the numerical system, are as follows:

Endospores not produced	200·
Facultative anaërobic	20·
Gelatin liquefied	1·
Acid and gas from dextrose	0·1
,, ,, lactose	·01
,, ,, saccharose	·001
Nitrates reduced without gas formation	·0003
Yellow chromogens	·00005
Feeble diastatic action on potato starch	·000002
No acid from glycerine	·0000003

Bacillus apiovorus then becomes 221·1113523.

This number approaches that for *Bacillus carotovorus* Jones (221·1113022), a form attacking carrots, etc., described by Jones[7],[8] in 1901 and more recently investigated by Harding and Morse[4].

The disease has not, up to the time of writing, been met with this season[1], nor has it been reported to us from elsewhere. No estimation of the damage done by the organism in the garden has been made, nor is it definitely known at what period of the year the plants are most subject to attack. The diseased plants examined were obtained during February and March of last year and it is to be expected that during the winter months, when the plants are not in active growth, they are most susceptible to bacterial injury.

Too little is known of the conditions inducing and favouring the development of the disease for remedies to be recommended, but the following are suggested as preventive measures:

(*a*) It is advisable not to grow celery in ground where the disease has been noticed the previous year. The organism will undoubtedly be washed into the soil, where it may live saprophytically or lie dormant until conditions are again favourable for it; in tube cultures it has been found to retain its vitality for several months, and, as under such conditions growth ceases within a few days, it must have remained quiescent during the greater part of the time.

(*b*) When the celery is stored remove all decaying or badly injured leaves.

(*c*) Burn promptly any plants showing a trace of heart-rot.

(*d*) Slugs, snails, or biting insects should be kept in check; these not only injure the plant, allowing the bacteria to reach the internal tissues, but disseminate the organisms by carrying them from one

[1] While this paper was in the press the disease reappeared in the College gardens.

plant to another. In some cases numerous mites were seen crawling over the decaying leaves, to which they were almost confined, though a few were found on healthy parts of the leaf.

In conclusion I desire to thank Mr E. S. Salmon, F.L.S., Head of the Mycological Department at the South-Eastern Agricultural College, for advice and help during the course of this investigation.

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Fig. 1.

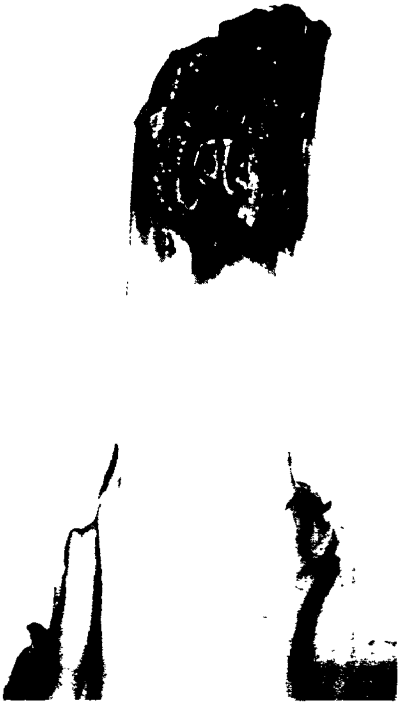

Fig. 2.

Fig. 3.

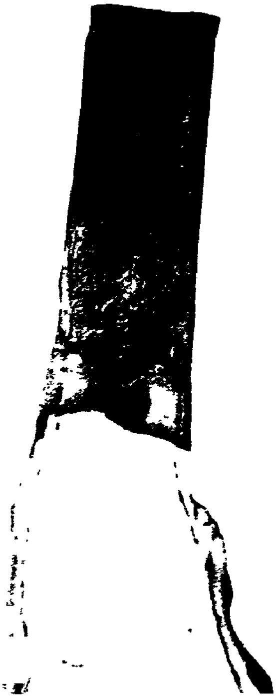

Fig. 4.

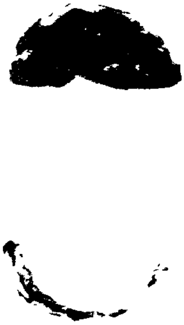

Fig. 5.

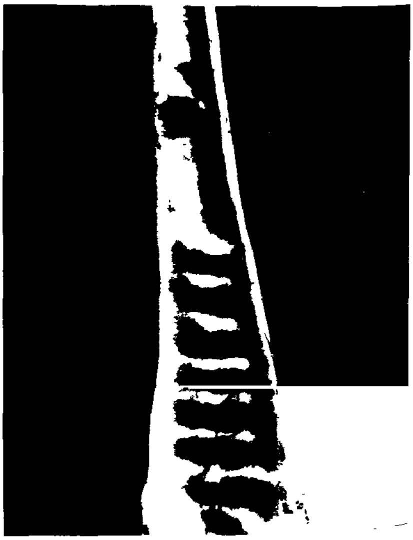

Fig. 6.

Fig. 7.

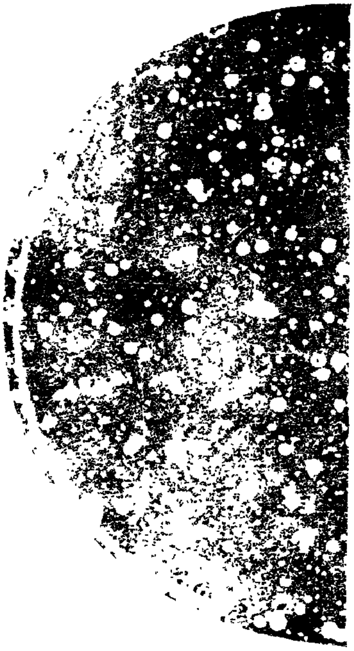

Fig. 8.

Fig. 9.

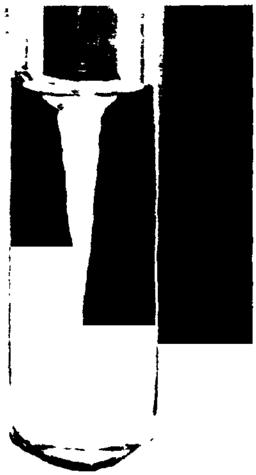

Fig. 10.

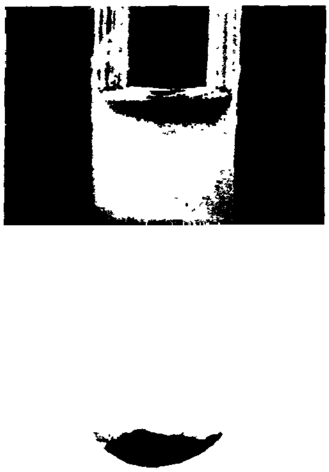

Fig. 11.

EXPLANATION OF PLATE II.

Fig. 1. A celery "heart" naturally infected, as seen after removing the leaves.

Fig. 2. The same cut longitudinally.

Fig. 3. A celery "heart" 18 days after artificial inoculation from a pure culture.

Fig. 4. The same cut longitudinally.

Fig. 5. A slice of celery cut into halves, one inoculated the other control.

Fig. 6. A celery petiole in which bacteria were found beneath the transverse ridges.

Fig. 7. A celery plant two days after inoculation of the leaf on the left; the leaf on the extreme right was the control (*i.e.* similarly wounded but not inoculated).

Fig. 8. Poured-plate culture (celery-extract agar) from a surface colony.

Fig. 9. Poured-plate culture (celery-extract agar) from a colony at the bottom of the agar.

(For description of the colony-forms shown in Figs. 8 and 9, see text.)

Fig. 10. Bouillon-gelatin stab culture, 20 hours old.

Fig. 11. The same culture as shown in Fig. 10, but 12 days old: gelatin completely liquefied with a turbid layer above and a dense sediment.

FURTHER OBSERVATIONS ON THE FUNGICIDAL ACTION OF BORDEAUX MIXTURES.

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In earlier papers (this *Journal*, vol. IV. pp. 69 and 76) we have detailed the experimental evidence which led us to conclude (1) that the view as to the fungicidal action of Bordeaux mixtures favoured by Pickering, viz. the liberation of copper sulphate by *atmospheric* carbon dioxide, is untenable; and (2) that contact between the fungus and the copper compound present in the mixture will account largely for its efficiency owing to a solvent action on the part of the organism under certain conditions. Pickering (this *Journal*, v. p. 273) has criticised our general conclusions and the deductions which we have drawn from certain of our experiments; and therefore before describing our further work on the subject, a brief reference to some of the points which he has raised is desirable.

Pickering now apparently accepts the view that the fungicidal action of Bordeaux mixtures cannot be attributed to the formation of copper sulphate, as he formerly maintained; for he attempts to demonstrate, by analogy with the results of experiments with metallic iron, that it is the slight solubility of the copper compound in water which is responsible for the action. Objection is raised to our experiments with spores on the ground that "the excretion of a solvent substance from them could only be established by examining their action on a copper compound which is really insoluble in water, and this Barker and Gimingham have not done, all their experiments having been performed with 10 CuO, SO_3 which is not insoluble." He has evidently overlooked the statement (p. 86, *loc. cit.*) that ordinary Bordeaux (containing the

insoluble compound 10 $CuO, SO_3, 3 CaO$) was also used in certain experiments. His comparison of the results with spores and those which can be obtained with metallic iron, on the assumed similarity of which he bases the whole argument of the remainder of his paper, therefore falls to the ground, since according to his own statement the compound 10 CuO, SO_3, 3 CaO does not itself act on iron. We have demonstrated that contact between it and the fungus can result in the death of the latter, even in the presence of a great excess of lime (as in ordinary Bordeaux mixture) under circumstances precluding the possibility that all the lime had been carbonated. This, on Pickering's own showing, therefore, can only be explained by the assumption of a solvent action. Further, the results of his experiments on the action of 10 CuO, SO_3 on iron rods in the presence of atmospheres containing different amounts of carbon dioxide show that the presence of that gas in quantities likely to occur under practical conditions *retards* rather than favours the solution and removal of the copper. (See Table, p. 278, *loc. cit.*)

He admits that there should be a differentiation between the action of carbon dioxide and of ordinary air on the Bordeaux precipitates, but suggests that "it is questionable how far such a differentiation is of importance from the point of view of practical spraying, for any fluid sprayed on to the leaves of trees will soon find itself bathed in an atmosphere of carbon dioxide evolved from the leaves themselves." This assumption of the presence of an atmosphere of carbon dioxide in the neighbourhood of leaves can surely not be accepted without proof, in view of the many factors which must tend to a rapid dispersal of the gas out-of-doors.

The following experiment bears on this point. All the leaves of a large strawberry plant in a pot were completely covered with a paste of the basic sulphate 10 CuO, SO_3. The plant was then placed in a bowl of water under a bell-jar in order to keep the atmosphere saturated. Under these conditions the action of any CO_2 respired by the leaves would presumably be at a maximum.

After three days, as much as possible of the transpired and condensed water on the leaves was shaken off into a small beaker. 2 or 3 c.c. of liquid were collected in this way. No dissolved copper could be detected in this liquid by the ferro-cyanide test. The leaves were then washed with distilled water and the wash water tested for copper, again with a negative result.

The experiment was repeated keeping the plant in darkness with the same result.

Again, stress is laid on the fact that a drop of Bordeaux mixture placed on a piece of paper soaked in potassium ferrocyanide solution gives the red colour of copper ferrocyanide as it dries up; and that further the red colour does not develop if the air in contact is deprived of its CO_2. We have specially referred to the action of atmospheric CO_2 (p. 74, *loc. cit.*). The ferrocyanide test is only given when the solid particles of the Bordeaux precipitate are in close contact with the paper, for no visible colour appears when ferrocyanide paper is dipped into the clear liquid above a Bordeaux precipitate, even if a current of air (containing the normal amount of CO_2) has been passed through the mixture previously for 5—6 hours. The results of such experiments do not, however, affect the question of the fungicidal action, because, as we have recorded (pp. 87 and 88 *loc. cit.*), spores will germinate and grow in the liquid in presence of the copper precipitate so long as they are not in actual contact with or sufficiently close to the actual particles[1].

Our experiments have failed, according to Pickering, to establish "the excretion of solvent matter from fungus spores." Our point was not so much that fungus spores in general possessed solvent properties, as that, whilst all living cells were capable of producing solvent material, the actual factor which determined the extent of the solvent action was the degree of permeability of the external cell wall concerned. Indeed, Pickering's description of the experiments with dried film cover-slip preparations to which we attached considerable importance (p. 88, *loc. cit.*) is quite inaccurate; and the results which we then obtained have been abundantly confirmed by the work shortly to be described.

Elsewhere in his paper, Pickering suggests that our results with ordinary Bordeaux in an atmosphere free from carbon dioxide were due to the fungicidal action of the excess of lime present, basing his statement on some results obtained by Foreman (this *Journal*, III. p. 400). Reference to the latter paper shows however that to claim "a conspicuous fungicidal action" on the part of the lime from these results is hardly justifiable.

Finally, the contention "that if fungicidal action depended on a solvent material exuded from the fungus, all copper compounds, or at any rate all the basic sulphates and carbonates, would be equally efficacious for a given weight of copper present, a proposition which is

[1] In view of this, we have throughout recorded as *nil*, any amount of copper less than that which will give a visible colour with ferrocyanide, although fully aware that in many cases by concentration of the liquid, copper might have been detected in solution.

contrary to all experience," assumes that the compounds are all equally susceptible to a given solvent (which is certainly not true as regards water) and at the same time ignores many other factors which are involved. For instance, there are manifest and well known differences in the solubilities and physical condition of the basic sulphates and carbonates of copper, to some of which Pickering himself has frequently drawn attention. These are amply sufficient to account for the observed differences in efficiency; and, indeed, from the practical point of view, the outcome of our work is to direct more attention to the importance of the physical properties of Bordeaux sprays than to their chemical nature.

We do not then consider that our main contention as to the cause of the fungicidal action of Bordeaux mixture has in any way been weakened by Pickering's criticisms. Indeed, in view of our further work we maintain even more strongly that the part played by the living cell in relation to the insoluble copper compound is the important point.

Experiments with Fungus Spores.

§ 1. In the first place the experiments with fungus spores described in our previous paper were repeated and amplified. The general results were precisely the same as those already recorded. The conidia of *Nectria ditissima*, whether the surrounding air be freed from CO_2 or not, are killed by Bordeaux mixtures when there is actual contact between the fungus spore or germ-tube and a particle of the copper compound; but, on the same coverslip and in the same film of liquid where there is no such contact, even though the conidia may be not more than their own length away from a copper particle, the organism is unharmed. The toxic action of the copper where it occurs is rapid, death of the cell ensuing in less than 12 hours. These results have been obtained with copper compounds of different degrees of insolubility including several basic sulphates and carbonates.

It is difficult to explain the results satisfactorily unless a solvent action on the part of the living cell is assumed. Pickering's explanation that death is due to absorption of minute traces of copper in solution in the continuous film of liquid covering the preparation absolutely fails to explain such cases. Still less can it explain the fact (also referred to previously) that the septate Nectria conidia may show death of one or more of the constituent cells, while the remainder may live and produce germ-tubes. Such localised effects indicate most

clearly the existence of a definite solvent action at the points concerned.

In some instances, it is true, groups of conidia, though not in actual contact with any particles of the copper compound, failed to make much growth and eventually died. It is probable that although a single cell may fail to secrete sufficient of the solvent to act at a distance from the copper, yet the combined efforts of a group of cells may well be successful.

Further experiments with thick-walled spores have confirmed the results with uredospores of *Puccinia* which were recorded in our earlier paper.

We have therefore as a result of these experiments definite evidence indicating a solvent action on the part of the living cell, the conditions determining the toxic effect being the quantity of the solvent substances passing out of the cell, the distance between the cell and the nearest particle of the copper compound and the amount of the latter within the sphere of action. In addition, the time factor must be taken into account. If the rate of growth of the cell and of the formation of new cells exceed the rate of production and absorption of the toxic dose of dissolved copper, death of the whole organism will not occur, although individual cells may succumb.

Experiments with other types of cells.

§ 2. In order to gain more light upon the general action of copper fungicides and, if possible, to obtain further evidence in support of our original conclusions, a number of experiments have been made upon the action of copper compounds on plant cells other than those of fungi. Before discussing these, we should like here to acknowledge the assistance given us by Mr J. W. Eves, who set up many of the experiments.

In the first place it was found that the root-hairs on the primary roots of seedlings could be utilised conveniently for such experiments; and in many respects they may be considered as types of cells comparable with the germ-tubes of fungus spores or the cells of actively growing hyphae. After a trial with several kinds of seedlings it was decided to work chiefly with those of the ordinary Broad Bean, since these proved to be very suitable; and the following observations refer to experiments with Broad Bean seedlings, unless it is otherwise stated.

When the radicle has grown to the length of an inch or so, root-hairs in all stages of development are to be found. The roots of some seedlings at this stage were placed in contact with Bordeaux mixture, great care being taken to avoid damage to the delicate root-hairs. The seedlings were then left overnight, and on the following morning it was possible to see quite clearly under a low power that in very many cases the protoplasmic contents of the root-hairs were dead. Often, not only had death occurred but the contents of the cells had become strikingly stained with a purplish blue colour, evidently due to the formation of some compound with the absorbed copper.

All the root-hairs were, however, not killed and some, as far as microscopic examination showed, not even injured. Assuming the existence of direct solvent action on the part of the cell, this is precisely the result to be anticipated, since the methods adopted would not cause particles of the basic copper sulphate to adhere to all of the root-hairs nor equal amounts to each. Hence the variability in the behaviour. If the injurious action was due to the production of soluble copper by agencies other than the living cell itself, the whole root-hair system should have been more or less equally affected instead of showing the extreme variations recorded.

§ 3. Other immersion experiments serve to complete the chain of evidence for the direct cell action hypothesis.

If the Bordeaux mixture precipitate is allowed to settle and the roots placed carefully in the supernatant clear liquid, on the day following the root-hairs are all apparently uninjured. (After several days, abnormal changes and death ensue; but as will be seen later, this is readily explained under the direct cell action hypothesis.) The case is even more striking if two seedlings are placed in the same vessel so that one has its root dipping into the precipitate at the bottom whilst the root of the other remains in the clear liquid above. After 24 hours many of the root-hairs of the former are killed, whilst those of the latter are all uninjured. These results have been confirmed with pea seedlings.

It is evident therefore that it is the direct contact between the thin-walled root-hair and the basic copper sulphate which causes the death of the cell within a few hours. In other words, there must be at work a reciprocal action between the cell and the insoluble copper compound when the two are in contact, which becomes inappreciable or is altogether absent when they are not in actual contact. The results are exactly analogous with those obtained with fungus cells. It is

therefore fair to conclude that living plant cells with walls of the type which permits ready passage of diffusible substances, are capable of exerting a direct solvent action on the insoluble copper compounds of Bordeaux mixture, provided the particles are sufficiently near to the cell.

§ 4. In experiments such as those just described, where the young root is brought into contact with the basic copper sulphate, not only do the root-hairs suffer in the manner indicated, but any part of the root in contact with the copper compound also becomes blackened. This is the case both with bean and pea seedlings. If the root tip rests in a pasty mass of the basic sulphate no further apical growth occurs. The portion of the primary root above the paste elongates and new secondary roots arise laterally from that region. These develop naturally until in their normal downward direction they strike the paste, when they suffer the fate of the primary root. The same kind of thing is repeated until in the end the whole root system is represented by a short knotted and blackened stump. Copper can be detected in the ash of the aerial parts of such plants.

§ 5. Again, if the paste of basic copper sulphate is applied to the young root in zones or bands, leaving intermediate portions untouched, the result is a blackening of the superficial cells on the treated areas within 24 hours, whilst the untreated areas remained unaffected.

§ 6. If bean seedlings are grown in water culture, using Bordeaux mixture, occasionally stirred, as the culture fluid, blackening of the roots ensues as in other cases of direct contact with the basic copper sulphate. On the other hand, if the Bordeaux mixture precipitate is allowed to settle before the introduction of the seedlings, the roots remain unaffected and continue in active growth for days in the clear liquid. When, however, such cultures are kept for some time, changes begin to appear. As the roots approach the layer of copper compound at the bottom of the vessel, the downward growth is checked and altered to a horizontal or even upward direction, away from the region of the copper. Under these circumstances the tips of the roots rarely actually penetrate into the deposit; and eventually after two or three weeks the roots in the clear portion of the liquid begin to show signs of blackening. Active growth ceases and finally the whole root system slowly acquires a knotted appearance, due to the attempted formation of secondary roots, the growth of which is almost at once arrested. Evidently, under the conditions of this experiment, no diffusion being possible beyond the limits of the vessel in which the plants were growing, there is an accumulation of the excreted products of the cells which, sufficient time

being given, finally reaches a concentration sufficient to act on the copper compound, even though it is situated at a slight distance.

§ 7. Many of these experiments were repeated in still more conclusive fashion, by placing the Bordeaux mixture inside a diffusion tube which was then partly immersed in a larger vessel containing the roots of the seedlings in the filtrate from Bordeaux mixture. Here there could be no possibility of direct contact between the roots and the particles of the copper compound. Control cultures without Bordeaux mixture were included. The results were the same as those of the experiment just described; the roots remained healthy and in active growth for some days, as in the controls, but eventually became discoloured and deformed.

§ 8. The same points were exceedingly well illustrated by experiments with mustard seedlings. Some strips of moist flannel were covered either completely or in isolated bands with the basic copper sulphate in the form of a paste. Mustard seeds were then sown fairly thickly over the whole surface and the flannel suspended with the ends dipping into water or Bordeaux mixture. The result was an excellent crop of seedling plants on the untreated areas which contrasted in a most marked manner with the ungerminated seeds and the few weak seedlings on the treated areas immediately adjoining. On the treated area, those seeds completely or almost completely immersed in the paste were killed before or immediately after germination. If however the micropyle of the seed was so situated that the radicle grew away from the paste, the total growth was sometimes sufficient to enable the seedling to withstand the shock when the radicle did eventually come into contact with the paste causing further apical growth to cease. Such cases were however rare, the great majority of the seeds getting scarcely beyond germination. The seedlings on the untreated area grew right up to the edge of the treated area; and equally good growth occurred if seeds were sown on the opposite side of a treated area, the seedlings then being removed from the paste only by the slight thickness of the flannel. Further, the seedlings grow freely and vigorously if distributed over the outside of a diffusion tube containing the basic copper sulphate paste.

§ 9. In all cases the injurious action of the paste on living cells is entirely local and cannot be explained as a result of the production of soluble copper by atmospheric agencies. The action is not limited to the basic copper sulphate formed in the "no-excess-lime" Bordeaux mixture ($10\ CuO, SO_3$), though this compound has been used in the

majority of the experiments. The same results are obtained with the compound present in ordinary Bordeaux mixture ($10\ CuO, SO_3, 3\ CaO$) and with copper carbonate. With the last mentioned substance, however, the solvent action of the cells is apparently less vigorous.

The Influence of the Nature of the Cell Wall.

All experiments dealt with up to this point have been concerned with the action of cells with walls more or less readily permeable, and serve to prove the correctness of the direct cell action hypothesis in the case of this type of cell. Our further work directs attention to the nature of the cell wall as a most important factor in the whole question of the relation of Bordeaux mixture to plant life.

§ 10. The effect of covering the seed coats of beans and of peas with a paste of the basic copper sulphate shows the behaviour with permeable cell walls of a somewhat different kind from those already discussed. In the case of the bean, discolouration of the outer seed coat occurs (confined to the areas in actual contact with the paste), but, under suitable conditions, germination takes place in normal fashion and so long as the radicle does not come into contact with the paste the young plant grows like that from an untreated seed. The blackening and discolouration is entirely restricted to the seed-coat and does not penetrate through it to the cotyledons. On the other hand, the seed-coat of the pea remains almost colourless but permits ready passage of copper to the cotyledons which acquire a blue-green colour. Germination is very much weakened or, if the time of contact with the paste is at all prolonged, entirely prevented. The thick fleshy coat of the bean retains all or practically all of the absorbed copper and the embryo itself escapes any toxic effect, whilst the thin semi-transparent coat of the pea readily transmits the absorbed copper to the cotyledons. If the seed-coat of the bean is removed before treatment, results like those with the pea are obtained.

§ 11. The action of cell walls of an entirely different type has been studied by an investigation of the interaction between the Bordeaux mixture and the leaf cells to which it is applied in practical spraying. The following account of experiments with apple and potato and other foliage is concerned with the behaviour of cells with impermeable or comparatively impermeable walls, such as the cuticularized outer walls of the cells of the leaf epidermis, when they are brought into contact with various copper compounds.

In this work with foliage it became apparent at a very early stage that trustworthy results would only be obtained when the portion of the leaf selected for the test possessed a cuticular surface absolutely free from injury of any kind. Emphasis must be laid upon this point, since the risk of abnormal results is very great if any part of the cuticle coming within the area of application of the fungicide has suffered damage. Great care is necessary in the selection of a suitable surface for the test on account of the rarity of occurrence of absolutely undamaged leaves on plants grown under ordinary outdoor or greenhouse conditions. In a very large percentage of cases the injury is so slight or so minute as to be invisible to the naked eye: but, although it may be only microscopic, it frequently suffices to vitiate the results. For this reason much of the work done with ordinary exposed foliage has been checked by corresponding experiments carried out with uninjured leaves obtained by growing the whole plant or individual branches enclosed in muslin screens from the time of the bursting of the buds, thus protecting the foliage from the possibility of damage by bruising or by insect attack. The following observations are confined to those cases in which we have satisfied ourselves that leaf injury has played no part.

§ 12. In the first place, in order to obtain evidence with regard to the extent to which the cuticularized walls of epidermal cells are impermeable, a number of healthy summer leaves of various kinds of apples (as far as possible free from visible injury) were immersed in copper sulphate solution (1, 5 and 10 per cent.) for periods varying from 5 minutes to 24 hours. It was remarkable how very little general injury to the surface of the leaves resulted. The damage was invariably restricted to separate areas starting from centres irregularly distributed over the surface of the leaf and could be increased by prolonging the immersion. The epidermis of the leaf as a whole is certainly not appreciably permeable: there is no general and simultaneous injury to the whole leaf surface. The appearance indicated that where damage resulted it was due to the penetration of copper to the inner thin-walled mesophyll cells of the leaf at certain isolated points. Under the microscope it was almost always possible to trace an original small injury as the centre of each area of damaged cells. Experience has shown that an absolutely uninjured apple leaf is rarely if ever to be found on a tree growing in the open, at all events after the early summer.

§ 13. Working at our suggestion, Mr S. P. Wiltshire, B.Sc., eventually got over the difficulty of finding an uninjured cuticular

surface to test by isolating small areas of leaves proved to be free from injury by microscopical examination. This was done by attaching glass ring cells of the type used for drop cultures to the surface of the leaf with vaseline and filling them with the liquid to be tested, thus confining the action to the area enclosed by the cell. By this method the uninjured epidermis of the apple leaf was shown to have a very remarkable power of resistance to the penetration even of corrosive liquids. Exposure to the action of 20 per cent. copper sulphate solution or of Perenyi fluid (3 parts alcohol, 4 parts 10 per cent. nitric acid, 3 parts 0·5 per cent. chromic acid) for several days caused no detectable injury to the upper surface of the leaves; on the lower surface a slight yellowing was observed which proved to be due not to any effect on the cuticle proper but to a discolouration of the walls of the hairs with which the lower epidermis is covered.

§ 14. Corresponding experiments to those with solutions of copper sulphate have been made with Bordeaux mixtures and with a paste of the basic sulphate 10 CuO, SO_3. As was to be expected, there was little or no effect upon the general surface of the leaf. Only at spots where an injury (natural or made purposely) allowed contact between the copper compound and the inner thin-walled cells was there any noticeable discolouration of tissue or "scorching[1]." On really uninjured leaves the hairs on the under surface were the only cells which showed the least trace of any absorption of copper; and this applies both to leaves treated with the basic sulphate paste and to those immersed in copper sulphate solutions. Several varieties of apples were tested in this way with no significant differences in the results. It should also be recorded here that Mr Wiltshire after a long series of examinations and measurements could find no sort of correlation between the thickness of the cuticle of the leaves of different varieties of apples and pears and their known susceptibility to scorching by Bordeaux mixture.

§ 15. One further point is of much interest in this connection. The observations described above no longer hold good when the experiments are repeated with autumnal foliage. After September distinct signs of an injurious effect of the copper treatment become apparent, both when Bordeaux mixtures or copper sulphate are used; and this effect is the more pronounced the later in the season the tests are made. General discolouration and scorching of the leaf surfaces, both

[1] Further work on the influence of injuries upon the "scorching" of foliage by Bordeaux mixtures and on the absorption of copper by plants is described in a paper shortly to be published in the *Annals of Economic Biology*.

upper and lower, quickly appear: and these are of quite a different character to those which occur locally at times during the summer experiments. Premature defoliation also follows, and the cuticularised epidermal cells of the leaves are found to be generally stained a blue green colour. The general effect is so uniform that it would seem to indicate some change in the nature of the cuticularized wall which renders it permeable and capable of a solvent action upon the copper compound. At the same time, since in the late autumn many leaves are likely to be considerably damaged, it is just possible that injury to the cuticle is sufficiently widespread to account for the results.

When the same experiment is repeated, using copper sulphate solution instead of Bordeaux mixture, a considerable amount of copper is absorbed and finds its way through the leaves and into the stem discolouring and killing all the cells through which it passes. Copper was easily detected by the ferrocyanide test in the ash of parts of the discoloured stem and of leaves remote from the parts actually treated with the copper sulphate solution[1].

§ 16. These experiments serve to show that the cuticularized walls of the epidermal cells of apple leaves in the summer stage are examples of typical impermeable walls. Their behaviour in relation to Bordeaux mixture as compared with that of unchanged cellulose walls, such as those of the root-hairs and roots of seedlings, may be considered analogous to that of certain thick walled resistant fungus spores in comparison with that of thin-walled germ tubes and actively growing hyphae. It is true that the impermeability of the wall of a fungus spore cannot be demonstrated so conclusively as that of a cuticularized epidermal wall; but it will probably not be disputed that the resistant spore wall is the fungus equivalent for a cuticle and therefore more or less impermeable so long as the spore is in a resting condition.

General Conclusions.

From the results here recorded it may be concluded that:

1. Living cells with readily permeable walls of the unchanged cellulose type or its equivalent are able to produce and absorb soluble copper from insoluble compounds such as the basic sulphates. (§§ 1—8.)

2. The area over which a single cell can exert the solvent action is

[1] Further work on the influence of injuries upon the "scorching" of foliage by Bordeaux mixtures and on the absorption of copper by plants is described in a paper shortly to be published in the *Annals of Economic Biology*.

imited by the size of the cell or, perhaps more accurately, by the quantity of the solvent diffusing from it. Groups of cells acting in conjunction may cause appreciable action over a wider area than an solated cell acting singly. (§§ 6, 7.)

3. The fate of the organism depends upon the relation between he amount of soluble copper produced and absorbed and the rate of growth of the organism. This is a significant point in connection with practical spraying, since it explains why there may be at times little check to the growth of a parasitic fungus after spraying, especially when he parasite has once gained a footing on the host-plant. (§ 8.)

4. Cells with walls of an impermeable character possess no such power of solvent action upon insoluble copper compounds. In the case of apple leaves only when there is injury to the cuticle sufficiently ecent for no occlusion to have taken place, or when there is some adical alteration in its nature, is soluble copper produced, with attendant "scorching" or local injury of the exposed thin-walled cells of the ubjacent tissues. (§§ 12—14.)

5. Under changed conditions, cells with normally impermeable walls may become permeable and capable of action upon insoluble copper compounds. The difference in behaviour of summer and autumn apple foliage would seem to be best explained in this way; and the change in the nature of the cell wall may perhaps reasonably be attributed to incipient death of the cells preparatory to leaf fall. This explanation fits in well with the fact that the hairs on the under urface of apple leaves (which are decadent cells) are affected by contact with the copper compound even in early summer, when the epidermal ells (being full of life and vigour) remain unattacked. (§ 15.)

It is evident therefore that the nature of the cell wall is the determinative factor in the matter of direct action of the cell upon the Bordeaux compounds. Comparison of the conclusions here stated with hose derived from our original work with cells of fungi and its epetition and extension described earlier in the present paper shows hat two distinct lines of work have led to identical results. The study f seedlings and foliage in relation to Bordeaux mixture has thus urnished the strongest support for our views as to the fungicidal action f that spray fluid.

STATISTICS OF BRITISH FEEDING TRIALS AND THE STARCH EQUIVALENT THEORY.

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DURING the last fifty years a very large number of investigations concerning the feeding of animals have been carried out. Many of them are of the greatest accuracy and interest in themselves, but perhaps the fact which strikes the student of the nutrition literature is the very small number of really far reaching generalisations which have been drawn from such a large amount of experimental results.

Perhaps the best known generalisation, at any rate the generalisation which we propose to discuss in the following pages, is that commonly known as the starch equivalent theory as applied to the production of increased live weight in fattening adult animals. Before proceeding further it will be well to give a brief account of the salient points of this theory as they were worked out by O. Kellner of Mockern[1]. An ox is kept for some time on a weighed ration which is so adjusted as to maintain the animal at constant weight. During this period the urine and faeces are collected and analysed, and from the figures so obtained it is possible to state the amounts of digestible protein and non-protein foodstuffs required to maintain the animal in nitrogen and carbon equilibrium. It is usual to state this maintenance ration in pounds of digestible protein and starch equivalent per 1000 lb. live weight. Starch equivalent for this purpose is reckoned by the formula :—starch equivalent = digestible protein $\times$ 1·25 + digestible fat $\times$ 2·3 + amides $\times$ 0·6 + digestible carbohydrates + digestible fibre. The generally accepted maintenance ration for a 1000 lb. ox on this basis is 6·35 lb. of starch equivalent which includes 0·6 lb. of digestible protein. When the diet of the ox has been carefully adjusted for maintenance in this

[1] O. Kellner, *Die Ernährung der landwirtschaftlichen Nutztiere*, Paul Parey, Berlin. Translation by W. Goodwin, *The Scientific Feeding of Animals*, Duckworth & Co.

way, he is kept for some time in a calorimeter and his heat evolution per day is found to be 10,800 Cals. The animal is then put into a respiration chamber and given a known weight of starch in addition to the previously ascertained maintenance ration. The requisite measurements are made to give a complete nitrogen and carbon balance from which it is found that a known weight of starch when added to a maintenance ration produces one quarter of that weight of fat in the animal's body, which corresponds to the utilisation of between 50 and 60 per cent. of the starch. The remaining 40 or 50 per cent. of the starch is converted into heat. Similar experiments in which known weights of cane sugar, protein or fat were added to the maintenance ration of an animal in a respiration chamber have shown that fat is formed in the following proportions[1]:

1 kilo of starch added to a maintenance ration forms 250 grams fat
1 ,, digestible fibre added to a maintenance ration forms 250 grams fat
1 ,, ,, protein ,, ,, ,, 235 ,,
1 ,, ,, fat ,, ,, ,, 474 to 598 ,,

From these figures it is possible to calculate how much fat should be stored in an animal receiving a known amount of any kind of food in excess of that required for maintenance, by adding together the amounts of fat which should be formed from the digestible protein, fat, carbohydrate and fibre contained in the known excess of the food above that required for maintenance. Such calculations can be checked by actual measurements of the fat formed when the food is given to an animal in a respiration chamber. The results of a number of such calculations are given below side by side with actual measurements[1].

1 kilo of	Fat calculated	Fat found	Found as °/o of calcd.
Cotton cake	201	197	98
Earth nut cake	189	189	100
Linseed cake	196	192	98
Hay	156	109	70
Wheat straw	104	21	20

From these figures it is clear that concentrated foods such as cakes made from oil seeds have the same value for fat production as the pure protein, fat and carbohydrate which they contain. This is far from being the case for bulky foods like hay or straw. Kellner allows for this fact in his method of calculating starch equivalents of foods for production. The following example will show his method.

From the figures given above for fat produced from 1 kilo of pure

[1] Kellner, *loc. cit.*

foodstuffs, factors are calculated which will convert other foodstuffs into the amount of starch equivalent for fat production. Thus the factor for protein will be 235/250, or 0·94; the factor for fat will vary from 474/250 or 1·9 to 598/250 or 2·4. The higher factor gives the value of fat in oil seeds and cakes made therefrom, the lower the value of fat in coarse fodders such as hay or straw. For cereal grains or other fodders of intermediate character the factor 2·2 is used. The factors for digestible carbohydrates and fibre are 1·0.

These values are used as follows:

Linseed cake.

Digestible protein	$28 \cdot 8 \times 0 \cdot 94 = 27 \cdot 2$ %
,, fat	$7 \cdot 9 \times 2 \cdot 4 = 19 \cdot 0$
,, carbohydrates	$25 \cdot 4 \times 1 \cdot 0 = 25 \cdot 4$
,, fibre	$4 \cdot 3 \times 1 \cdot 0 = 4 \cdot 3$
	75·9

Assuming that all the foodstuffs of linseed cake have the same value as pure foodstuffs for fat production, the starch equivalent of linseed cake would be 75·9. But the experiments quoted above show that linseed cake produces only 98 per cent. of the full calculated amount of fat which it should produce on this assumption. To get its true starch equivalent for fat production Kellner therefore multiplies 75·9 by 98 and divides it by 100. The true starch equivalent for fat production is therefore 74·4, or 98 per cent. of full value.

Wheat straw.

Digestible protein	$0 \cdot 2 \times 0 \cdot 94 = 0 \cdot 2$ %
,, fat	$0 \cdot 4 \times 1 \cdot 9 = 0 \cdot 8$
,, carbohydrates	$13 \cdot 3 \times 1 \cdot 0 = 13 \cdot 3$
,, fibre	$20 \cdot 4 \times 1 \cdot 0 = 20 \cdot 4$
	34·7

On the assumption that all the foodstuffs of wheat straw are of the same value to the animal as pure foodstuffs, the starch equivalent of wheat straw should be 34·7. The figures for the actual amount of fat produced by a known weight of wheat straw show that only 20 per cent. of the full calculated value of the foodstuffs of wheat straw is utilised for fat production by the animal. The true starch equivalent of wheat straw according to Kellner should therefore be $34 \cdot 7 \times 20/100$, or 7·0.

Kellner has assigned starch equivalents determined on this basis to all the common foods, to the number of over three hundred. His figures are readily available in the appendix of his book or in the translation[1].

[1] Kellner, *loc. cit.*

The appendix quoted gives also the percentage digestibility of all the foodstuffs of the common foods, and standard rations for animals of all kinds and all ages. Kellner's figures are intended to provide a definite basis for arranging rations for the production of meat, milk or work, since they give a quantitative relation between the amount of food converted into its starch equivalent and the amount of fat, work or milk which it may be expected to produce.

The question naturally arises—can one predict with any approach to accuracy what amount of fat, work, or milk will be produced by a diet supplying a known amount of starch equivalent above that required for maintenance? British experimenters who have tested this point do not find that the increases produced by various diets are proportional to the excess of starch equivalent above that required for maintenance provided by the diets as calculated on the basis of Kellner's figures[1]. But it is obviously impossible to throw over the starch equivalent theory, based as it is on such a considerable amount of accurate experimental work, because it is not supported by a few isolated feeding trials. It seemed to us therefore that valuable results might be obtained by a statistical examination of the results of as many feeding trials as are conveniently accessible. Fortunately for our purpose there appeared recently admirable summaries of feeding trials with oxen and sheep compiled for the Highland and Agricultural Society by H. Ingle[2]. These summaries comprise the results of about 200 trials with oxen, and 200 trials with sheep, all calculated by the author on a uniform basis. The experiments summarised include practically all those recorded in British periodicals down to the year 1907. For the sake of convenience of reference, in case our conclusions should lead to discussion, we have confined our examination to the trials recorded by Ingle, though many results have been published since 1907.

The first point to which we gave attention was the starch equivalent of swedes. Roots are not grown for fodder on the continent on anything like the scale adopted in British farming. In Norfolk, for instance, an ox put up for winter fattening receives in his daily ration from 100 to 150 pounds of roots. Kellner's figures for the starch equivalent of roots are based on very few experiments and in those the rations used were very small. It is quite likely therefore that the discrepancy between British experimental results and the starch equivalent theory may be

[1] W. Bruce, *Reports of Feeding Trials*, Edinburgh and East of Scotland Agricultural College.

[2] *Journ. Highland and Agric. Soc.* 1909–10.

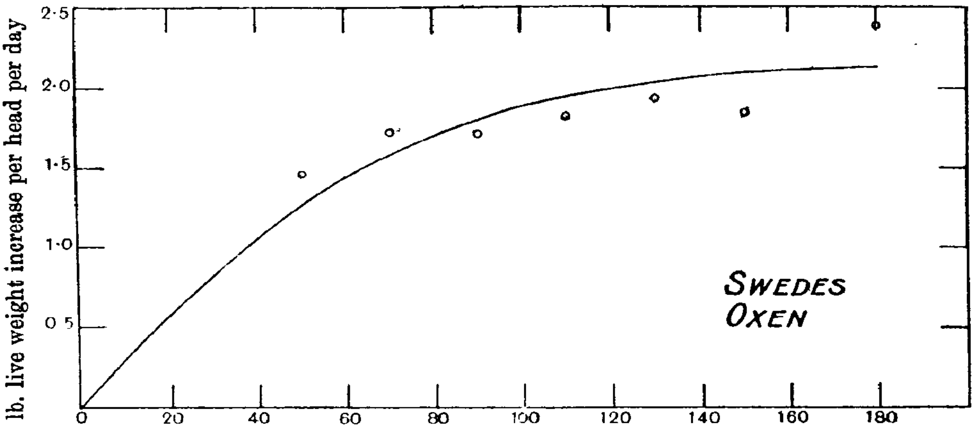
due in part to the fact that Kellner's figures for the starch equivalent of roots are not accurate when the roots are used in such large amount as is usual on British farms.

In order to obtain information on this point we extracted from Ingle's summary of feeding trials with oxen the results of all the trials in which swedes formed a large proportion of the diet. We then proceeded to calculate from Kellner's tables of the percentage of digestible foodstuffs in average samples of common foods the starch equivalent of the constituents of the diet other than swedes, using for this purpose the formula quoted above, page 233. This quantity was then compared with the amount of starch equivalent required for the maintenance of an animal of the weight recorded. If it was exactly equal to the maintenance ration, all we had to do was to note how many pounds of swedes produced the recorded increase in live weight. If it exceeded the maintenance ration the excess in terms of starch equivalent was converted into the corresponding amount of swedes by multiplying it by 100/7·5, 7·5 being the starch equivalent of 100 lb. of swedes according to Kellner. If the starch equivalent of the diet excluding swedes was not enough to provide maintenance, the deficit in terms of starch equivalent was converted into the corresponding amount of swedes by multiplying by 100/9, 9 being the starch equivalent of swedes for maintenance. The correction thus determined was then added to or subtracted from the amount of swedes in the ration, and the corrected amount of swedes thus found was noted together with the recorded increase in live weight. In this way the following figures were obtained.

Corrected weight of swedes in ration above maintenance per day	Number of experiments	Average live weight increase produced	Probable error of average
40 to 60 lb.	3	1·46	—
60 to 80 ,,	4	1·73	—
80 to 100 ,,	14	1·72	0·082
100 to 120 ,,	17	1·83	0·061
120 to 140 ,,	13	1·93	0·078
140 to 160 ,,	8	1·85	0·048
over 160 ,,	3	2·39	—

These figures indicate that the increase in live weight was greater as the amount of swedes above maintenance was increased, but the increase in live weight was not nearly so rapid as the increase in swedes. This is well shown in the Fig. 1 in which the ordinates represent

increase in live weight and the abscissae amounts of swedes in diet above maintenance.



lb. swedes (corrected) per head per day above maintenance. Oxen

FIG. 1.

The figures and the curve seem to indicate that the amount of increase in the live weight of an ox produced by increasing amounts of food above that required for maintenance is subject to the ordinary law of diminishing return. The importance of this point is that if it can be established, in other words if successive increases in the amount of food above that required for maintenance produce successively diminishing increases in live weight, then it follows that starch equivalents must be assigned on a sliding scale on which the starch equivalent diminishes as the diet is increased.

This point can be illustrated by calculating from the above figures what can only profess to be rough approximations to the percentage utilisation of the swedes for different rations. In order to do this it is necessary to make two assumptions. In the first place Lawes and Gilbert[1] have shown from their comparison of the composition of oxen in store and fat condition that the average composition of the increased live weight put on during fattening is 67 per cent. dry matter, chiefly fat, and 33 per cent. water. Assuming that this figure is approximately true for all the animals under experiment we can calculate the weight of fat in the live weight increase produced by each ration of swedes. The second assumption is that 1 gram of starch equivalent yields in the animal 3·76 Cals., and 1 gram of fat 8·8 Cals. Applying these

[1] *Journn. R.A.S.E.* Vol. xxi. 1860, 433.

figures we get the following values for the percentage utilisation of swedes when used in varying quantities above maintenance.

Corrected weight of swedes in ration above maintenance	Percentage utilisation
50	51
70	43
90	33
110	29
130	26
150	21

The figures receive some support from the fact that Kellner's average figure for percentage utilisation is 50 per cent. which is about the same as the utilisation of swedes in the British trials when used in small quantities. They also agree with figures directly determined at the Norfolk Agricultural station in the winter of 1913—14. Two lots of 10 oxen of similar weights were fed on just sufficient cake and chaff to supply maintenance. The one lot received in addition one bushel of swedes or 46 lb., the other lot 3 bushels or 138 lb. During the course of the experiment which lasted for 28 days the amounts of swedes consumed were respectively 12,600 lb. and 37,800 lb., and the increases in live weight 339 and 704 lb. Working out these figures as before we find that when the amount of swedes was only 46 lb. over maintenance the percentage utilisation was 47. With the larger ration which provided 138 lb. over maintenance the percentage utilisation fell to 32.

In the experiments on which Kellner based his starch equivalents the amounts of food added to the basal ration varied very considerably. It is possible therefore to plot the results of these experiments as we have plotted those of the British experiments. For convenience in doing so we have extracted the figures for all the experiments quoted in Table VII of the appendix to *The Principles of Animal Nutrition*, by H. P. Armsby[1]. The figures are given there in terms of the heat values of the amount of food digested above that required for maintenance and of the increased live weight produced. These figures were classified and averaged with the following results.

[1] Wiley & Sons, New York, 1906.

Heat value of food above maintenance	Heat value of increase in live weight	Percentage of excess food utilised as increased live weight
1595	646	41
3423	1704	50
4417	1987	45
6331	3040	54
7520	3162	42
8728	4792	55
9391	4443	47
10288	4979	48
11560	6022	53
12344	6380	52
13531	7533	56
15129	7422	49
16489	8960	54
17373	8653	50
19635	9962	50
	Average.........	50 ± 1

From this it appears that of the foods used in these experiments, namely meadow hay, oat straw, wheat straw, extracted straw, molasses, gluten, starch and pea nut oil, there is direct proportionality over a very wide range of diet between the excess of food above that required for maintenance and the amount of increase produced. In all cases approximately 50 per cent. of the excess of food expressed as calories above maintenance requirements was utilised as increase in live weight. This point is well shown in Fig. 2, in which Kellner's figures are plotted.

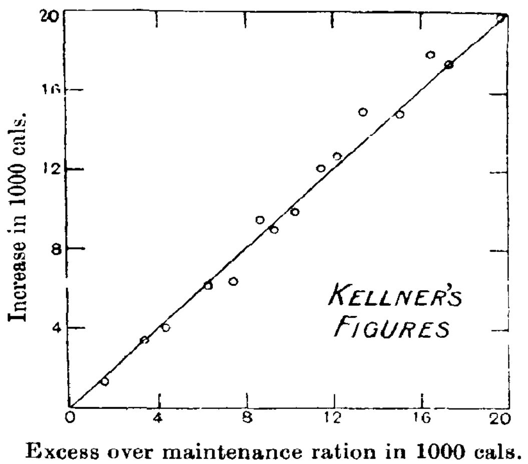
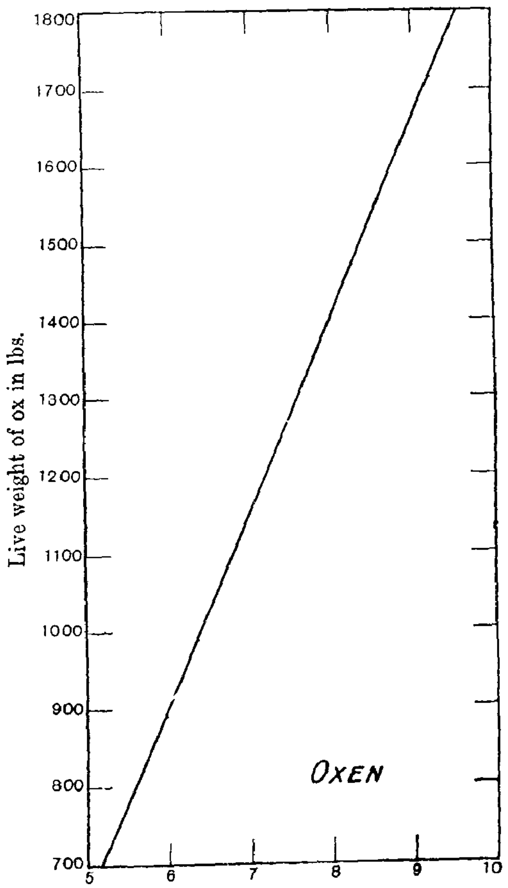


Fig. 2.

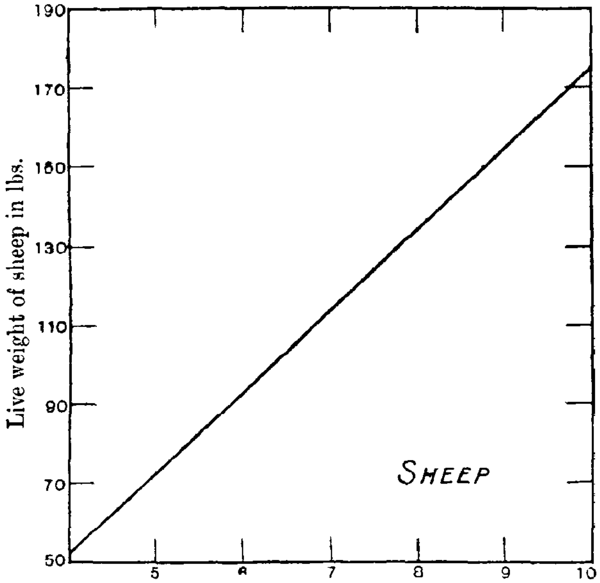
This result is evidently at variance with the estimates of the utilisation of swedes which we have made in the preceding pages, which indicated that the percentage utilisation of the food decreased as the amount of food above that required for maintenance is increased. Before attempting to seek an explanation of this discrepancy we recalculated the starch equivalents of all the diets for both oxen and sheep given in Ingle's tabulated statement already referred to, using the formula quoted on page 233. All the trials quoted in the tables were included except a few on the accuracy of which Ingle throws some doubt. The figures were then classified and averaged with the following results. The amounts of starch equivalent required for maintenance were read off from the annexed diagrams, Figs. 3 and 4, the live weights



lb. starch equivalent per head per day required for maintenance

FIG. 3.

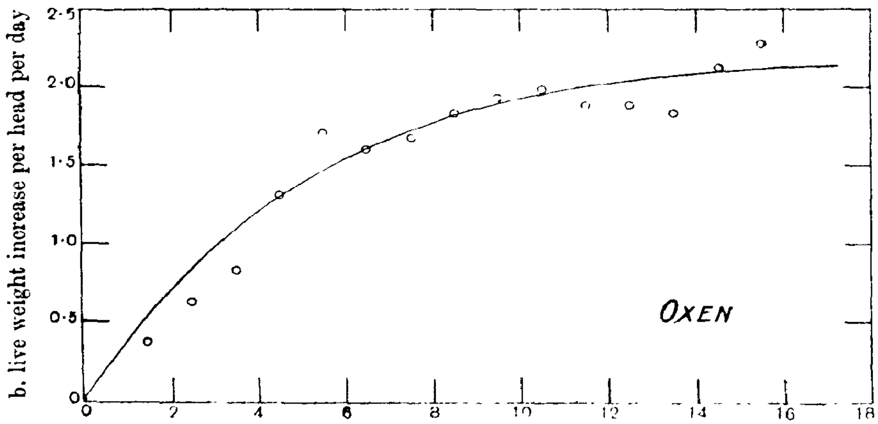
of the animals used in each experiment being given in the tables. The diagrams were constructed from figures taken from Kellner's *Ernährung*



lb. starch equivalent per head per week required for maintenance

FIG. 4.

der landwirtschaftlichen Nutztiere. The results are plotted in Figs. 5 and 6 which show graphically the relation between the starch equivalent of the diet above maintenance requirements, and the live weight increase.



lb. starch equivalent per head per day above maintenance

FIG. 5.

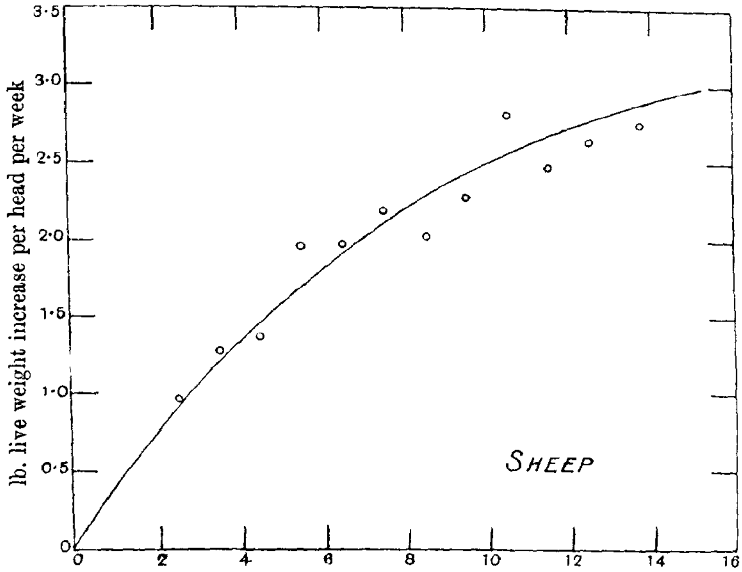


FIG. 6.

lb. starch equivalent above maintenance	No. of observations	lb. increase in live weight, mean	Probable error of mean
	Oxen		
6— 7	14	1·618	0·063
7— 8	27	1·674	0·048
8— 9	37	1·833	0·046
9—10	29	1·931	0·056
10—11	12	1·992	0·107
11—12	15	1·885	0·068
12—13	15	1·897	0·045
13—14	15	1·840	0·054
	Sheep		
3— 4	11	1·298	0·115
4— 5	14	1·377	0·117
5— 6	14	1·965	0·105
6— 7	25	1·991	0·082
7— 8	33	2·191	0·058
8— 9	34	2·016	0·083
9—10	10	2·276	0·118
10—11	11	2·801	0·111

To compare these figures with Kellner's it is necessary to convert both the food and the increase into heat units. This has been done by multiplying the weight of food in pounds by 454 to convert it into grams

and the product by 3·76 to convert this into Calories. The latter figure gives the energy which can be obtained from 1 gram of starch equivalent by an ox. The increased weight is assumed to be 67 per cent. fat. Its fat content is thus calculated, and multiplied by 454 and 8·8, the latter figure giving the amount of energy which an ox can obtain from 1 gram of fat. From these figures the percentage utilisation of the food for the production of increase is easily calculated.

lb. starch equivalent above maintenance: oxen per day, sheep per week	Percentage utilisation	
	Oxen	Sheep
3— 4	—	58 ± 5
4— 5	—	48 ± 5
5— 6	—	56 ± 3
6— 7	39 ± 2	48 ± 2
7— 8	35 ± 2	46 ± 2
8— 9	34 ± 1	37 ± 2
9—10	32 ± 1	37 ± 2
10—11	30 ± 2	42 ± 2
11—12	26 ± 2	—
12—13	24 ± 1	—
13—14	21 ± 1	—

Examination of these figures shows at once that they agree with the figures already given above for the utilisation of swedes. The larger the amount of food above the maintenance ration, the smaller proportion utilised. The figures are comparable with those quoted above for Kellner's experiments, at any rate as regards range of diet. The smallest ration, 0·5 lb. per head per day of starch equivalent above maintenance corresponds to about 1000 Calories. The largest ration of 13·5 lb. per head per day of starch equivalent to about 23,000 Calories. The somewhat higher percentage utilisation by the sheep seems to suggest that too small an amount was subtracted from the total ration for maintenance. This is not unlikely for the data for the maintenance ration of sheep are very scanty.

Comparison of the figures given above with those quoted from Kellner's experiments make it quite clear why British experimenters do not obtain results in their feeding trials which agree with Kellner's starch equivalent theory. Kellner's starch equivalents are based on figures which indicate a direct proportionality between the amount of food consumed above the maintenance ration and the live weight increase produced. The figures which we have calculated from Ingle's

tables of British feeding trials indicate clearly and with certainty that the percentage utilisation of food decreases as the diet is increased, each successive increase giving a smaller return in live weight increase than the last.

We have considered many possible explanations of the divergence between these British figures and Kellner's. Some of the points we have investigated seem worthy of record. In the first place we tested the reliability of the average results on which our conclusions are based by working out the probable errors of the averages from the variation of the separate figures. In the case of the experiments with oxen the decrease in percentage utilisation is from 39 per cent. to 21 per cent. and the probable error of these figures is at most 2 per cent. There can be no doubt therefore that there is a significant decrease in the utilisation of increasing amounts of food by oxen. In the sheep experiments the decrease is from 58 per cent. to 37 per cent., a decrease of 21 per cent. The probable error of these figures is about 3 per cent., or only about one seventh of the decrease. Again we can only conclude that the decrease is a real one. Kellner's figures too indicate a real proportionality, for his figure for the average utilisation of 50 per cent. has a probable error of less than 1 per cent. Both sets of figures therefore are sufficiently accurate, and the divergence is a real one.

The next point we investigated was the range of the diets in Kellner's experiments and in the British experiments under discussion. The figures we have given above show that the explanation of the divergence does not lie in varying range. Kellner's experiments dealt with diets varying from 1500 to 19,000 Calories above maintenance requirements. The British experiments include diets ranging from 1000 to 23,000 Calories per head per day above maintenance requirements.

A further possibility seemed to be that whilst Kellner's figures are based on experiments in a respiration chamber in which the carbon fixed in the form of increased weight was accurately determined by means of a carbon balance, the British results were obtained in ordinary feeding trials in which the utilisation is calculated from the increase in live weight by a factor depending on a very small number of experiments carried out at Rothamsted many years ago. This factor may be assumed to give the proportion of fat in the total increase of weight as a lean animal becomes fat, but it may fail to give with accuracy the proportion of fat in successive increases as the animal slowly fattens from the lean condition. There is some evidence to show that the first additions of live weight increase to a lean animal may contain as much

as 50 per cent. of water and only 50 per cent. of fat, whilst the last additions of weight to the animal when it is nearly ripe, as the butcher would say, may be almost entirely fat. In other words the factor for converting live weight increase into fat in order that the utilisation may be calculated ought possibly to increase as the animal fattens from 0·5 to 1·0. In the case of the oxen this would increase the lowest utilisation with the highest diet from 21 per cent. to 31·5 per cent., which is still significantly below the highest percentage utilisation of 39 per cent., and much lower than Kellner's average figure of 50 per cent. The maximum possible variation in the factor evidently fails to reconcile the two sets of figures, and we conclude therefore that the explanation of the divergence is still to seek.

It next occurred to us that the divergence might be due to the fact that whilst Kellner's experiments were confined to the measurement of the carbon balance for a short period including the formation of only a few pounds of fat, the period of the British experiments was some 4 or 5 months during which the animals laid on several hundredweights of live weight increase. It appeared to us quite possible that there might be a direct proportionality between food and increase in the early stages of fattening whilst the animal is still comparatively lean, that in fact the law of diminishing return might not begin to come into operation until the animal attains a notable degree of fatness. None of Kellner's experiments were carried out on animals in this condition. In all the British experiments the animals were fed to the extreme condition of fatness at which they are said to be ripe for the butcher. It is common knowledge to anyone who has weighed oxen at various stages of the fattening process that the rate of increase in live weight of the animals begins to decrease as they approach this condition of ripeness. The suggestion therefore seemed to be worth investigating. Accordingly we classified all the experiments with oxen according to the period of feeding, and then worked out the percentage utilisation of the excess of starch equivalent above maintenance for experiments of different periods. In classifying, the total number of experiments was divided into four classes each including between 40 and 50 experiments. The percentage utilisation was then worked out for the experiments included in each period by the method already explained. The results are given below.

Length of experiments in days	Percentage utilisation
20— 80	32
80—100	31
100—120	33
120—180	32

Evidently the length of time of the experiment has on the average no effect on the percentage utilisation of the food. The reason of this probably is that in some of the shorter experiments animals already half fat were used, and there was accordingly low utilisation throughout. Certainly some of the figures for the short experiments show very small utilisations. These are balanced by others of the short experiments showing very high utilisations probably due to the fact that lean oxen were fed for a short period which would include only the early stages of fattening, whilst the utilisation is very good. Thus for the individual experiments in the shortest period the utilisation varies from 52 per cent. to 13 per cent. Under these circumstances the length of the experiment is not a measure of the extent to which the fattening process was pushed, and the figures for utilisation in the experiments of different lengths are not likely to throw any light on the question under discussion. We therefore determined to attack the question in another manner. In some of the experiments the original details of which are readily available, notably a series carried out by one of us[1] for the Norfolk Chamber of Agriculture in the years 1896 to 1899, we were able to calculate the percentage utilisation during each successive month of the whole series of experiments. The figures which are given below are most instructive. They are calculated from the average results for each successive month of the two lots of animals whose ration included respectively linseed cake, and both linseed cake and common cotton cake.

Percentage utilisation of starch equivalent above maintenance.

First month	Second month	Third month	Fourth month
39	34	31	15

The average utilisation for the first month is very good; probably the utilisation for the first week would approach Kellner's 50 per cent. It does not fall off very much during the second and third months, but in the fourth month the falling off is very striking indeed. By the end of the fourth month the animals were approaching the ripe condition.

In our opinion the divergence between Kellner's figures and the results of British feeding trials is due to this fact, that in Kellner's experiments really fat animals were never used, whilst in British feeding trials the animals were almost invariably fed until they were ripe for the butcher. It is during the last stages of fattening that the

[1] T. B. Wood, *Journ. of Bd. of Agr.* Vol. VI.

utilisation falls off to the greatest extent. The reason the more liberal diets were not so well utilised on the average appears to be that on a liberal diet the animal gets into the ripe condition more quickly, and the ripening stage forms a greater proportion of the whole feeding period in such cases than when a less liberal diet is given. This seems to explain both the smaller utilisation in British trials than in Kellner's, and the fact that in British trials the utilisation decreases as the amount of food is increased above maintenance requirements, whilst in Kellner's experiments it was constant within wide limits of daily ration above maintenance. Possibly the lower utilisation in the British trials might be due to our having taken the fat as forming in all cases 67 per cent. of the live weight increase, but this would not explain the decreasing utilisation with increasing diets.

Our final conclusion on this point is that, whilst Kellner's starch equivalents may give a fairly accurate measure of the amount of fat production to be expected from various foods in the early stages of fattening, they fail to do so in the later stages, because as fattening approaches completion the law of diminishing return asserts itself, and a given amount of starch equivalent produces less and less fat as time goes on and the animal gets ripe. Kellner's starch equivalents, too, fail when very liberal diets are used because here the animal very quickly reaches that stage of fatness at which the law of diminishing return begins to make itself felt.

Under these circumstances is it possible to give a fixed value to the starch equivalent of various foods in the strict sense in which Kellner meant, namely that the starch equivalent of any food was that amount which would produce as much fat as 100 lb. of starch? We can see no reason why the relative fat producing value of starch and any other food should vary as the fattening process advances, why for instance the law of diminishing return should not apply equally to starch and roots. If this is so it may be possible to draw up a sliding scale for the utilisation of starch equivalent varying with the conditions which we have shown above to influence the utilisation of the diet. Such a scale could be arrived at by the use of the annexed curve, Fig. 7, which shows the relation of starch equivalent of swedes to the excess above the maintenance ration. At present we have not sufficient data for giving a curve to show the relation between the value of the starch equivalent and the period of fattening.

The figures on which we have based our remarks are the average of large numbers of experiments. When the figures for the experiments

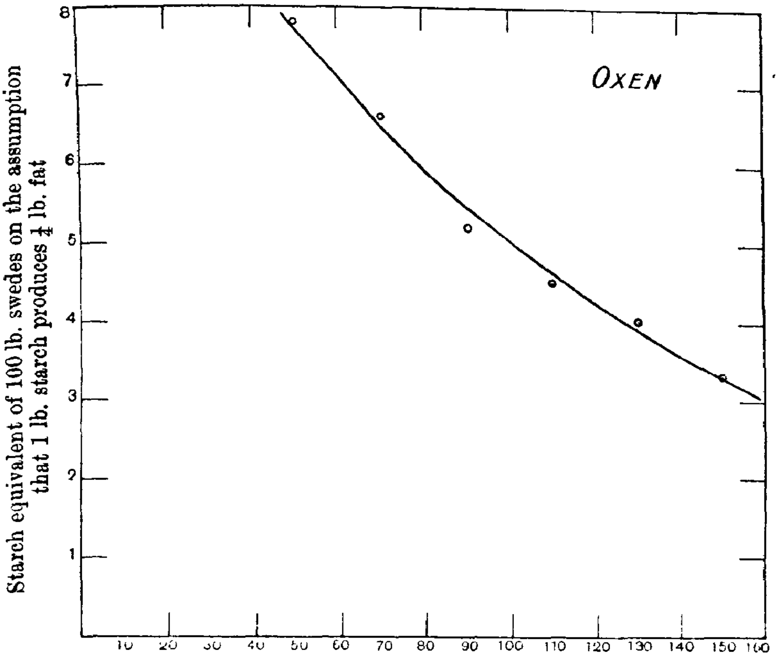


FIG. 7.

are considered separately the extent to which they differ is very striking. To illustrate this point we have calculated the standard deviations for both the oxen and the sheep. The figures are given below as percentages of the mean live weight increases.

Standard deviations of single experiments as percentage of live weight increases.

lb. starch equivalent above maintenance	Oxen	Sheep
3—4	—	41
4—5	—	45
5—6	—	29
6—7	21	30
7—8	22	22
8—9	22	35
9—10	23	23
10—11	26	18
11—12	20	—
12—13	14	—
13—14	16	—

These figures require some consideration. One of us[1] in discussing the accuracy of agricultural experiments has shown that the probable error in the increase of one animal on a fattening diet is 14 per cent. of the live weight increase. This corresponds to a standard deviation of 21 per cent. This figure refers to animals of the same age and breed fed together under the same conditions. The experiments tabulated by Ingle include on the average five animals in each experiment with oxen, and 15 in each of the sheep experiments. Taking 21 per cent. as the standard deviation of a single animal, then if all the conditions of all the experiments were the same the standard deviation of the average of five oxen should be $21 \div \sqrt{5}$ or about 10 per cent., and that of the average of 15 sheep only $21 \div \sqrt{15}$ or about 5 per cent.

In the experiments with oxen the standard deviations come out about twice as high as they should if all conditions were the same in all experiments. This is of course due to the fact that conditions varied greatly. For instance different breeds and different ages of animals were used, the animals in different experiments were variously housed, and the temperature at which the animals were kept varied greatly from year to year and from place to place. All these causes contribute to increase the deviation between experiments, and their magnitude may be inferred from the fact that the standard deviation was about twice as high as might have been expected if all conditions had been kept the same throughout.

The same remarks apply equally, or even more strongly, to the sheep experiments, for there the standard deviations are in some cases eight times greater than they should have been if all conditions remained the same throughout all the experiments. This is probably due to the fact that additional causes of variation are introduced in the case of sheep by the varying amounts of wool produced by different breeds and by the greater variation in temperature to which animals fed in the open air in winter are exposed.

But apart from varying conditions the amount of variation in efficiency as fat producing machines among animals of the same breed fed under identical conditions is remarkable. As we have mentioned above the standard deviation under these conditions is 21 per cent. of the live weight increase, and the probable error in the increase of one animal 14 per cent. Taking 1·833 as the average increase per head per day produced by oxen consuming the most popular ration of 8—9 lb.

[1] T. B. Wood and F. J. M. Stratton, This *Journal*, Vol. III. 417, and T. B. Wood, *Journ. of Bd. of Agr. Suppl.* No. VII. 15, Nov. 1911.

starch equivalent above maintenance, it follows that one animal in four must increase more than 2·1 lb. and one animal in four less than 1·6 lb. per head per day. A diet supplying 8·5 lb. of starch equivalent per day above maintenance possesses a heat value of about 25,400 Cals. per day. An average sized ox on such a diet would retain in his body 8400 Cals. if he laid on 2·1 lb. of fat, or 6400 Cals. if he made only 1·6 lb. of fat per day. Subtracting these values from the total heat value of the diet, it appears that out of every four oxen fattened one would be likely to give out less than 17,000 Cals. per day and one more than 19,000 Cals. A difference of over 8 per cent. in the rate of heat evolution should therefore be quite common in fattening oxen. On examining figures for the average increases produced by numbers of individual oxen on fattening diets it is quite common to find animals which increase as much as 3 lb. and as little as 1 lb. per head per day. These figures correspond to heat evolutions of 17,000 Cals. and 22,000 Cals. per head per day, the difference between which is about 25 per cent. Provided therefore that there is no appreciable difference in the amount digested from similar diets by different animals there should be a difference of the order of 25 per cent. in the rate of heat evolution of what the farmer would call good and bad "doers." Investigations are at present in progress in Cambridge on the variation of digestibility according to the individual peculiarities of the animal and the amount of the diet, and on the possibility of estimating the rate of heat evolution from animals of known fattening capacity by measuring the difference between their skin temperatures and the temperature of the surrounding air.

SKIN TEMPERATURE AND FATTENING CAPACITY IN OXEN.

By T. B. WOOD, M.A.,
Drapers Professor of Agriculture,
AND A. V. HILL, M.A.,
Fellow of Trinity College, Cambridge.

In the preceding paper, attention is drawn to the fact that wide differences occur between individual animals in their capacity for converting food into fat. It is shown, for instance, that an individual ox on a fattening diet may increase in live weight as much as 3 lb. a day or as little as 1 lb. a day. The former would give out 17,000 Cals., and the latter 22,000 Cals. per day, a difference in heat evolution of about 25 per cent.

The suggestion was made that it might be possible to establish a relation between the fattening capacity of an animal and its skin temperature, because the difference between the skin temperature and the air temperature must bear a definite relation to the heat evolution. We have tested this suggestion by measuring the skin temperatures of 18 oxen which had been for some time on a fattening ration at the Norfolk Agricultural Station. The measurements were made on March 25th, when the animals had been for more than two months on a diet of 6 lb. of mixed linseed and cotton cake, 135 lb. of roots, and about a stone of straw chaff per head per day. The measurements were taken by means of a thermopile of simple construction. Two wires, one of copper the other of constantan, each about 8 feet long, were soldered at one end on to a small tin plate which was placed in contact with the animal's skin, at the other end to a large tin plate which was allowed to hang free so as to take up the temperature of the air. Leads were taken off to a galvanometer.

Preliminary experiments showed that the skin temperature was constant throughout a large area over the ribs behind the shoulder

blade. The animals were driven as quietly as possible into the cage of the weighbridge. The tin plate, protected from draught by a muslin hood, was pressed firmly on to the hair covering the skin behind the shoulder blade. The needle of the galvanometer was watched until the deflection became practically constant, when the reading was recorded. Constancy was generally reached in between 5 and 10 minutes. The animal's weight was then recorded, and its rate of increase calculated from a weighing taken 21 days previously on March 4th. The figures are given below :

Mark on animal	Average live weight increase per week during last 3 weeks	Reading of galvanometer in scale divisions*
No. 3	25	65
1	24	73
7	22	61
2	21	73
4	15	64
10	15	82
9	15	65
13	15	71
Average of good "doers"	19	69
No. 20	14	79
5	12	65
15	12	80
14	11	77
12	10	67
Average of moderate "doers"	12	74
No. 6	8	72
19	6	83
11	5	81
18	5	79
17	0	77
Average of bad "doers"	5	78

* 1 scale division = 0·3° C. very nearly.

In the table the animals are divided into three classes: good "doers," which had increased in live weight more than 2 lb. per head per day during the last three weeks; bad "doers" which had increased less than 1 lb. per head per day during the last three weeks; moderate

"doers" which had made increases intermediate between these extremes. The average figures for each class indicate that the animals which were making large increases had a lower skin temperature than those which were making small increases, the difference amounting to 9 scale divisions, which corresponds to about 3° C. It is noticeable that every animal in the class of bad "doers" had a higher skin temperature than the average of the eight good "doers." The figures in the separate classes are not quite satisfactorily uniform, but some of the exceptions are readily explicable. For instance the high temperature of the skin of No. 10, was almost certainly due to his having exerted himself considerably whilst being driven from the yard to the weighbridge. The experiment can only be regarded as preliminary, but the results seemed to be of sufficient promise to be worth recording. With further refinements the method may be a valuable means of investigating fattening capacity.

METHODS OF ESTIMATION OF CARBOHYDRATES. III.

THE CUPRIC REDUCING POWER OF THE PENTOSES—XYLOSE AND ARABINOSE.

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(Rothamsted Experimental Station.)

Received March 21st, 1914.

IN the scheme of analysis of plant extracts described in a former paper (Davis and Daish, *J. Agric. Sci.*, 1913, **5**, 437), before it is possible to calculate the proportion of dextrose and laevulose an allowance must be made for the pentoses present; it therefore became necessary to ascertain the exact value of the cupric reducing power of these sugars under the standard conditions adopted—namely those defined by Brown, Morris and Millar[1]. Values of the cupric reducing power of xylose have been given already by Stone[2], Weiser and Zeitschek[3], and of arabinose by the latter workers and by Ost[4]: but as they were obtained under conditions different from those specified by Brown, Morris and Millar, they are unsuitable for the present purpose, and it became necessary to make a fresh series of determinations. Xylose and arabinose are the only pentoses which are at all readily obtainable and in the present paper attention is limited to these.

XYLOSE.

Kahlbaum's "pure xylose" was used; the final value of the specific rotatory power when mutarotation was complete, was $[\alpha]_D^{20\cdot0} = +18\cdot76^\circ$ ($c = 5\cdot1420$) This value was not perceptibly changed by two recrystallisations from alcohol. After such treatment, it was obtained in beautiful,

[1] *Trans. Chem. Soc.*, 1897, **71**, 72.
[2] *Ber.*, 1890, **23**, 3796.
[3] *Pflüger's Archiv*, **93**, 98; *Landw. Versuch.*, **53**, 219.
[4] *Ber.*, 1890, **23**, 3003.

slender needles $[\alpha]_D^{20\cdot0} = 18\cdot78°$ ($c = 5\cdot0755$). The results of series A were obtained with the original material; those of series B with the recrystallised xylose.

Series A.

5·1387 grms. xylose (dried to constant weight at 96° in vacuo over phosphorus pentoxide) were dissolved in water and the solution made up to 100 c.c. at 15° C. 20 c.c. of this solution, representing 1·0277 grm. xylose, were diluted to 250 c.c. at 15° C. and the reducing power determined with different volumes of this solution under the conditions specified by Brown, Morris and Millar.

TABLE I.

Volume taken	Wt. of xylose	Mean CuO *	Grms. CuO per 1 grm. xylose
50 c.c.	0·2055	0·4687	2·281
30 ,,	0·1233	0·3016	2·446
25 ,,	0·1028	0·2541	2·472
20 ,,	0·0822	0·2081	2·532
10 ,,	0·0411	0·1100	2·676

* The values given are the mean of at least two closely concordant results.

Series B.

Recrystallised xylose $[\alpha]_D^{20\cdot0} = 18\cdot78°$ (final value).

(*a*) 2·5361 grms. (dried in vacuo at 96° over phosphorus pentoxide) were dissolved in water, the solution being made up to 50 c.c. at 15° C.; 10 c.c. of this solution (= 0·5072 grms. xylose) were diluted to 250 c.c. at 15° C.

TABLE II.

Volume taken	Wt. of xylose	Mean CuO *	Grms. CuO per 1 grm. xylose
50 c.c.	0·1014	0·2506	2·471
30 ,,	0·0609	0·1550	2·545
20 ,,	0·0406	0·1049	2·584
10 ,,	0·0203	0·0543	2·675

* The values given are the mean of at least two closely concordant results.

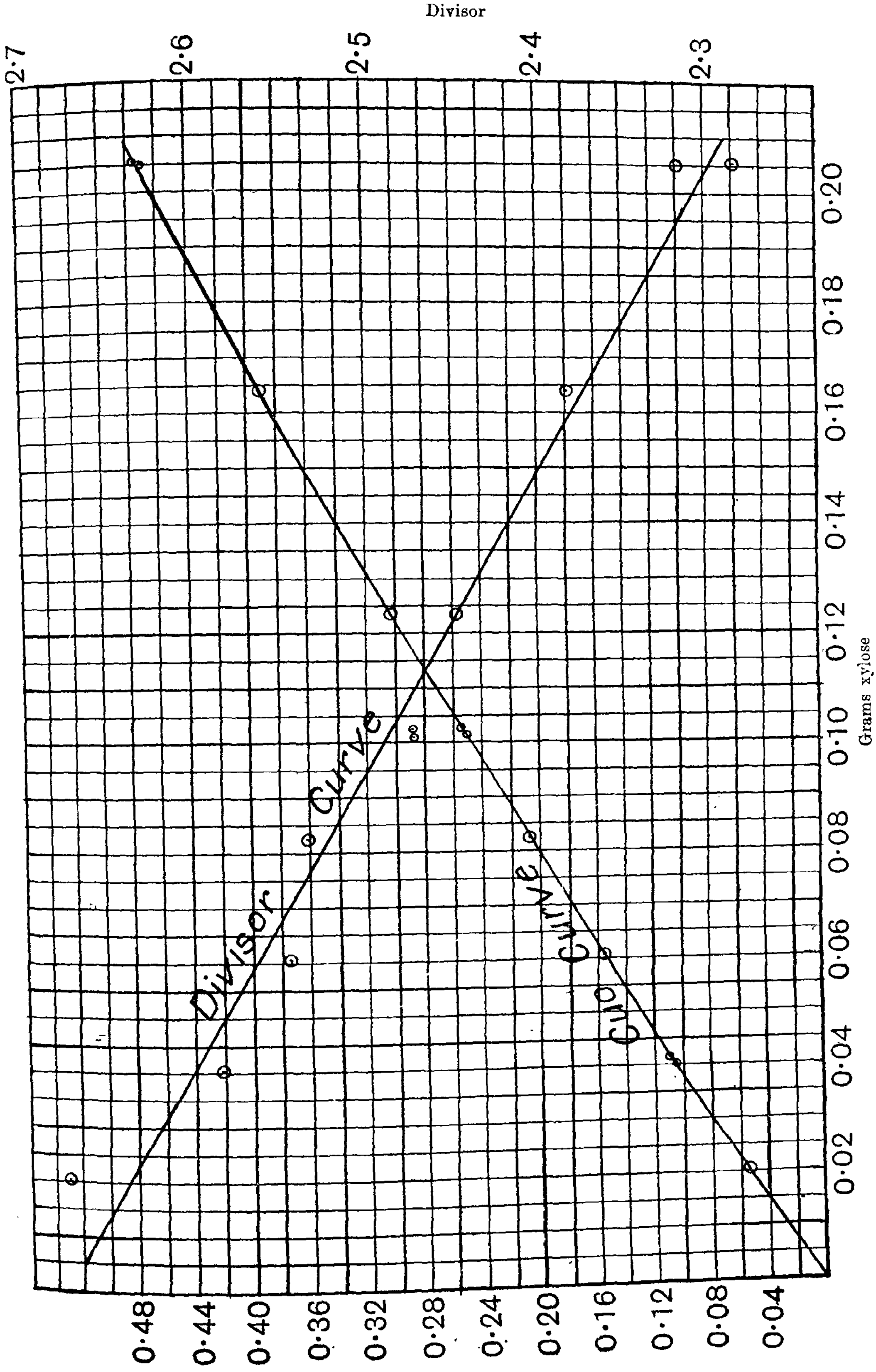

Divisor
2·7
2·6
2·5
2·4
2·3
Divisor Curve
CuO Curve
0·02
0·04
0·06
0·08
0·10
0·12
0·14
0·16
0·18
0·20
Grams xylose
0·48
0·44
0·40
0·36
0·32
0·28
0·24
0·20
0·16
0·12
0·08
0·04

Fig. 1.

(*b*) 0·8194 grm. dry xylose dissolved in water and the solution made up to 100 c.c. at 15° C.

TABLE III.

Volume taken	Wt. of xylose	Mean CuO	Grms. CuO per 1 grm. xylose
25 c.c.	0·2049	0·4744	2·315
20 ,,	0·1639	0·3901	2·380

Fig. 1 shows the curve obtained by plotting the reducing power expressed as CuO against the corresponding weights of xylose; the "Divisor Curve" gives the value of the ratio $\frac{\text{weight of CuO}}{\text{weight of xylose}}$ for each weight of xylose. To obtain the weight of xylose corresponding to any given weight of CuO, it is best to use the "divisor curve" and to divide the weight of CuO obtained by the value of the divisor corresponding

TABLE IV. *Reducing power of xylose under the conditions specified by Brown, Morris and Millar.*

Milligrams xylose	Grms. CuO	Calculated divisor	Divisor from curve
10	0·0280	2·800	2·656
20	0·0540	2·700	2·638
30	0·0798	2·660	2·620
40	0·1040	2·600	2·602
50	0·1300	2·600	2·581
60	0·1540	2·583	2·563
70	0·1790	2·557	2·545
80	0·2030	2·537	2·526
90	0·2260	2·511	2·508
100	0·2490	2·490	2·490
110	0·2720	2·473	2·471
120	0·2940	2·450	2·453
130	0·3160	2·431	2·433
140	0·3380	2·414	2·415
150	0·3600	2·400	2·397
160	0·3810	2·381	2·378
170	0·4020	2·365	2·360
180	0·4230	2·350	2·341
190	0·4440	2·337	2·322
200	0·4640	2·320	2·304

to this weight of CuO. In Table IV are given for successively increasing weights of xylose the values of the corresponding divisors; Column 3 shows the divisor calculated from the actual weights of CuO

obtained from the "CuO curve" and Column 4 the more correct divisor obtained from the "divisor curve." When the amount of xylose is small, there is a considerable difference between the two values, such as is inherent in the nature of the method; with larger weights of xylose the agreement between the two series becomes quite satisfactory.

ARABINOSE.

The specimen of arabinose obtained from Kahlbaum was found to contain a small amount of impurity; this became evident on comparing the specific rotatory power of the original material with that of the same material after recrystallisation from 80 % alcohol. The product obtained after two recrystallisations, however, gave a constant value of $[\alpha]_D$ and the same value was obtained with a specimen of arabinose specially prepared from gum arabic by hydrolysis with 2 % sulphuric acid according to the method described by O'Sullivan (*Trans. Chem. Soc.*, 1884, **45**, 41; 1891, **59**, 1029). The results in series A were obtained with Kahlbaum's arabinose, twice recrystallised $[\alpha]_D^{20\cdot0} = +102\cdot14°$ ($c = 6\cdot8064$); the results in series B with the specially prepared arabinose recrystallised until the specific rotatory power was constant $[\alpha]_D^{20\cdot0} = 102\cdot33°$ ($c = 4\cdot669$).

Series A.

3·4040 grms. arabinose (dried to constant weight at 96° in vacuo over phosphorus pentoxide) were dissolved in water and the solution made up to 50 c.c. at 15° C. 25 c.c. of this solution (= 1·7020 grms. arabinose) were diluted to 250 c.c. at 15° C. and the reducing power determined with different volumes.

TABLE V.

Volume taken	Wt. of arabinose	Mean CuO	Grms. CuO per 1 grm. arabinose
30 c.c.	0·2042	0·4846	2·373
25 ,,	0·1702	0·4156	2·442
20 ,,	0·1362	0·3399	2·496
10 ,,	0·0681	0·1757	2·580
2·5 ,,	0·0170	0·0445	2·630

Series B.

Arabinose specially prepared from gum arabic. 2·3352 grms. arabinose (dried in vacuo at 96° over phosphorus pentoxide) were dissolved in water and made up to 50 c.c. at 15° C. 20 c.c. of this solution (= 0·9341 grm. arabinose) were diluted to 250 c.c. at 15° C.

TABLE VI.

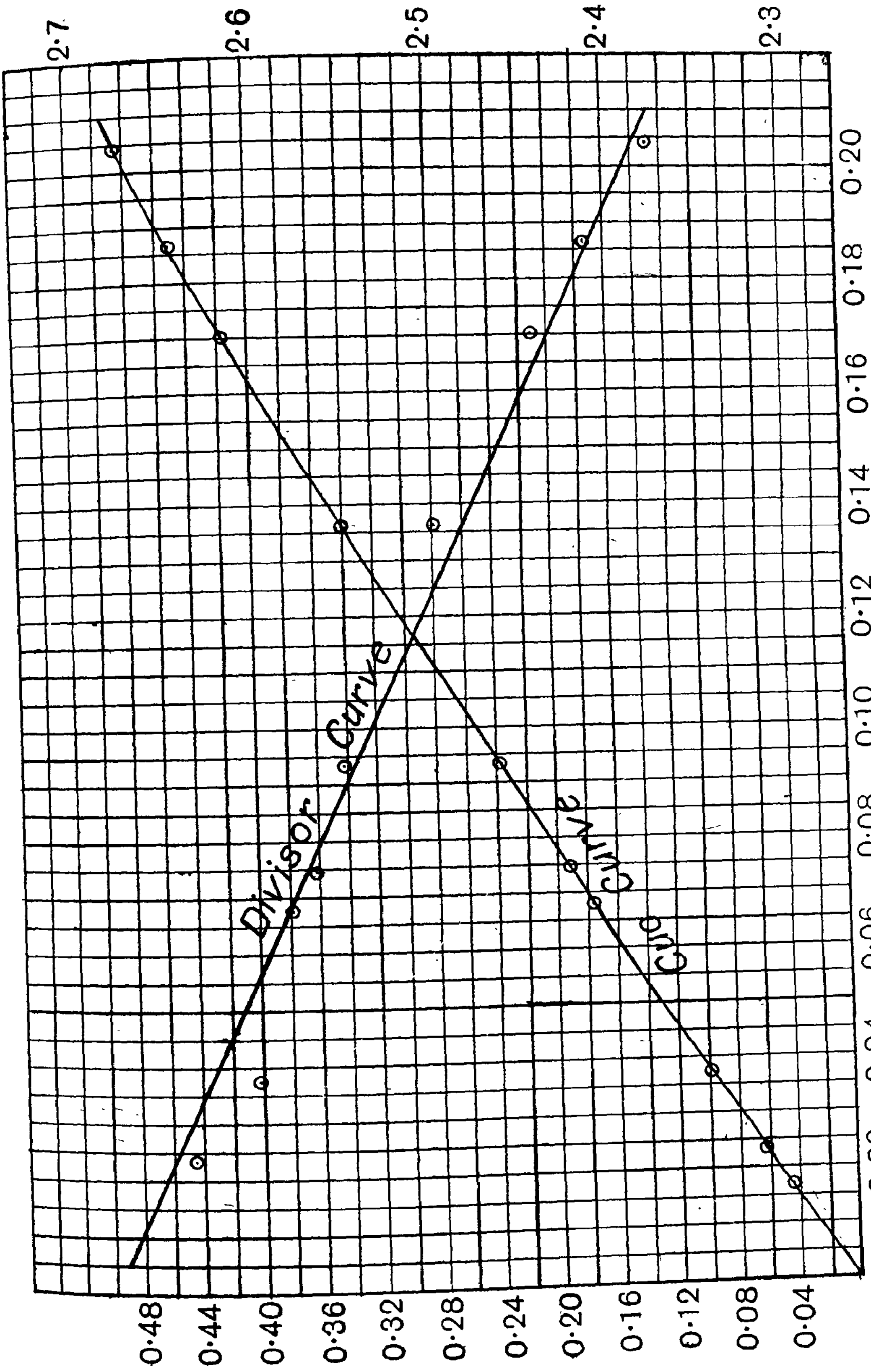
Volume taken	Wt. of arabinose	Mean CuO	Grms. CuO per 1 grm. arabinose
50 c.c.	0·1868	0·4505	2·412
25 ,,	0·0934	0·2383	2·551
20 ,,	0·0748	0·1920	2·567
10 ,,	0·0374	0·0973	2·602
—	0·0234	0·0617	2·637

Fig. 2 shows the values obtained in both series plotted in the form of a curve as in the case of xylose. Table VII gives the values of the "divisor" for different weights of arabinose, at intervals of 10 milligrams.

TABLE VII. *Reducing power of arabinose under the conditions specified by Brown, Morris and Millar.*

Milligrams arabinose	Grms. CuO	Calculated divisor	Divisor from curve
10	0·0270	2·700	2·669
20	0·0540	2·700	2·654
30	0·0804	2·680	2·640
40	0·1064	2·660	2·625
50	0·1320	2·640	2·610
60	0·1570	2·617	2·595
70	0·1820	2·600	2·581
80	0·2060	2·575	2·566
90	0·2300	2·556	2·551
100	0·2540	2·540	2·536
110	0·2780	2·527	2·521
120	0·3020	2·517	2·507
130	0·3248	2·499	2·492
140	0·3476	2·483	2·477
150	0·3700	2·467	2·461
160	0·3920	2·450	2·447
170	0·4140	2·435	2·432
180	0·4360	2·422	2·417
190	0·4570	2·405	2·403
200	0·4780	2·390	2·381

Divisor



Grams arabinose

Fig. 2.

It will be seen on comparing Tables IV and VII that the reducing powers of arabinose and xylose are almost identical. For practical purposes, when working with the unknown pentoses in plant extracts, it is probable that no large error will be incurred by taking as the divisor the average value for arabinose and xylose corresponding with the weight of CuO dealt with. It is interesting to note that the reducing powers of arabinose and xylose differ only very slightly from that of dextrose; thus the divisors for these three sugars for 100 milligrams of sugar are respectively 2·536, 2·490 and 2·538.

ON SOME FACTORS CONTROLLING FERTILITY IN DOMESTIC ANIMALS.

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INTRODUCTION.

THE fertility of domestic animals is a subject of ever-increasing importance to the stock-breeder for, with the improvement of our breeds of live-stock, there has come in many cases lessened fertility and often sterility. The Royal Commission on horse-breeding found that in mares about 40 % of those selected for breeding failed to produce foals.

Pearl[1] has recently shown that the variation in fecundity (eggs produced) in various breeds of the common fowl is not anatomical; that a fertile strain of fowls does not necessarily contain more oocytes in the ovary than an infertile strain. He concluded that the fecundity of fowls depends on a physiological factor causing the oocytes to develop and grow. The same applies to mammals, for the human ovary at puberty is said to contain 20,000 oocytes, a number sufficient for the shedding of more than 40 ova each month of sexual life. In mammals it is known that many of these oocytes atrophy at some stage of their development, but this has not yet been described in the common fowl. Heape[2] found that in rabbits (which only ovulate after copulation) the mature follicles atrophy when the buck is withheld. Sandes[3] also found that the presence of the corpus luteum in the ovary causes the surrounding follicles to become atrophic. Atrophic follicles have been described

1 Pearl, *Jour. Exp. Zool.* Vol. XIII. 1912.
2 Heape, *Proc. Roy. Soc.* B, Vol. LXXVI. 1905.
3 Sandes, *Proc. Linnean Soc. N.S.W.* Vol. XXVIII. 1903.

in the ovaries of many species of animals (Benthin[1], Schottländer[2], etc.). Heape[3] and Marshall[4] who investigated the fertility of sheep came to the conclusion that several factors influenced the number of ova shed during the heat period: among others, service early in the breeding season and the practice of "flushing." These conditions they thought were favourable to the ripening of the oocytes and prevented atrophy of the follicles.

Certain of Heape's results however could not be explained as a simple result of the number of follicles which ripened, for, with Dorset Horn ewes he found that when bred to a ram of their own breed they were less fertile than when crossed with a Hampshire ram. Darwin[5] quotes somewhat similar cases in pigs, the most striking being one from Nathusius who inbred a strain of pigs (Large Whites) for three generations. "One of the latest sows, produced, when paired with her own uncle (who was known to be productive with sows of other breeds) a litter of six and a second time a litter of only five weak young pigs. This sow was then mated to a boar of a small black breed (this boar when mated to sows of his own breed produced from seven to nine young). Now the sow of the large breed, which was so unproductive when paired with her own uncle, yielded to the small black boar in the first litter 21 and in the second 18 young pigs." This agrees with the popular opinion that inbreeding reduces fertility and that an out-cross brings it back again. Other investigators have noticed a reduced fertility on inbreeding—Ritzema Bos[6] with rats and von Guaita[7] with mice. Castle and others[8] however, working with the fly Drosophila, found that inbreeding probably reduces very slightly the productiveness, but that productiveness may be maintained if selection is made from the more productive families; an out-cross however increased the fertility.

Since ovulation occurs spontaneously in the pig and is not influenced by copulation the decreased fertility on inbreeding must be due to other

[1] Benthin, *Arch. f. Gynaek.* Bd. XCIV. 1911.

[2] Schottländer, *Arch. f. mikros. Anat.* Bd. XLI. 1893.

[3] Heape, *Jour. Roy. Agric. Soc.* Vol. X. 1899.

[4] Marshall, *Trans. Highland and Agric. Soc.* Vol. XX. 1908.

[5] Darwin, *The Variation of Animals and Plants under Domestication*, Vol. II. Popular edition, London, 1905.

[6] Ritzema Bos, *Biol. Centrbl.* Bd. XIV. 1894.

[7] von Guaita, *Ber. d. Naturf. Gesellt.*, Freiburg, Bd. X. 1898.

[8] Castle, Carpenter, Clarke, Mast and Barrows, *Proc. Amer. Acad. of Arts and Sci.* Vol. XLI. 1905–6.

reasons than the number of follicles rupturing at the heat periods. Heape suggested that in the sheep the decreased fertility when inbred might be due to some sort of abortion. Stephenson[1] attributes many cases of sterility to the male. "Bulls are often the unsuspected cause of abortion. Some bulls, apparently healthy, vigorous and good servers, —bulls, too, that have been good stock getters—appear to lose their procreative powers. Cows that are served by them are seldom settled and if so, often abort. There is a want of vitality, varying in degree, in the spermatozoa, which prevents the ovum or foetus reaching maturity, this causing abortion at different stages of gestation."

Mr R. Assheton has told me that in rabbits he has observed a number of cases in which the fertilized ova get implanted in the wall of the uterus but do not develop beyond a certain stage.

Material.

In the present paper an attempt has been made to determine the factors controlling the number of young produced at birth by counting the number of corpora lutea and foetuses in pregnant animals at various stages of gestation.

The animals examined were rabbits, pigs, and a bitch. Since pregnant animals are seldom killed it is rather difficult to obtain specimens except in the case of the rabbit.

The rabbits. Does were bought from dealers in odd lots. When a number had been collected they were kept under the same conditions of feeding etc. for some time, and then were allowed to copulate with two different bucks at short intervals on the same day. The does were each kept in separate hutches and the bucks kept in another house so that there was no chance of superfoetation or formation of corpora lutea by copulation subsequent to the first impregnation.

The pigs. These were collected at various times from butchers, and except for two cases nothing whatever is known of their history. These sows (VI and VII) were put up to fatten and were put to the boar a short time before killing. A record of their treatment was kept at the University Farm, Cambridge. I am greatly indebted to Mr Mackenzie for this material.

The bitch. This was a mongrel killed during the latter part of pregnancy.

[1] Stephenson, *Jour. Roy. Agric. Soc.* Vol. XXI. 2nd series, 1885.

In all the animals examined it is quite easy from simple inspection to count the number of corpora lutea in the ovary during the first half or three quarters of their duration. Later, when they have begun to atrophy, one can be certain of finding them by making incisions across the ovary with a knife. In pregnant animals the uterus was slit longitudinally between the endometrial folds so that the foetuses could be seen and counted.

The factors controlling fertility will be divided into two groups:—

1. Factors controlling the number of ova shed (the fecundity of Pearl).
2. Factors controlling the number of embryos which develop to reach maturity.

1. *Factors controlling the number of ova shed.*

In all probability it is as regards this factor that species and breeds vary in the matter of fertility. It seems to be a complicated physiological factor which can be modified to a certain extent by improving the nutrition of the ovary. Thus Heape and Marshall found that "flushing" causes increased fertility in ewes and that this is probably due to the maturation of more follicles.

Marshall states that in the life of the individual there is a curve of fertility which rises at first rapidly and then falls again gradually later in life. With fowls however Pearl[1] finds that the greatest egg production is in the first year. It is well known that the number of offspring produced by a young animal breeding for the first time is below normal. Minot[2] has shown that with guinea pigs the size of the litter increases with age during the first sixteen months of their lives. I am indebted to Mr P. G. Bailey for the following figures which show that this is true also of the rabbit. The average size of the first litter was $5{\cdot}58 \pm {\cdot}32$, that of the second was $7{\cdot}25 \pm {\cdot}41$ and the third $7{\cdot}08 \pm {\cdot}38$.

Wallace[3] states that sows ought to rear 6—8 pigs in their first litter and 10—12 or more in each after-litter.

The number of corpora lutea occurring at a time in the ovaries of several young and old sows have been counted and are given below (Table I). It would seem that the lower fertility of young sows is due to a smaller number of ova being shed.

[1] Pearl, *Jour. Exp. Zool.* Vol. XIII. 1912.
[2] Minot, *Amer. Jour. of Physiol.* Vol. XII. 1891.
[3] Wallace, *Farm Live Stock of Great Britain*, Edinburgh, 1907.

TABLE I.

	Average no. of corpora lutea in ovaries	Range of numbers
18 young sows	14·3 ± ·39	11 to 19
9 old sows	19·77 ± 1·26	13 to 24

2. *Factors controlling the number of embryos which develop to reach maturity.*

On examining the ovaries of sows killed just after the heat period one is struck by the large number of corpora lutea present. From the figures given above it will be seen that the average number of ova shed or corpora lutea produced at a heat period is twenty, while the average litter of young pigs is about twelve. According to Rommel[1] and Surface[2] the average litter is less in American breeds; in Duroc-Jerseys 9, and Poland-Chinas 7·5.

The uteri and ovaries of pregnant animals (pigs, rabbits and a bitch) have been examined to determine the cause of this phenomena. The results show that (*a*) some ova are shed (at the time of conception) of which no trace can be found in the uterus and that (*b*) other ova although developing in the uterus to a certain extent never come to maturity but atrophy at some stage of their development. For the purpose of description these two conditions will be treated separately.

In case (*a*) where no trace of the ova could be found at the time of examination, the ova may have been lost by wandering in the body cavity instead of being caught by the Fallopian tubes. This could not occur in the carnivora where the fimbriated end of the Fallopian tube completely surrounds the ovary; probably a systematic examination of pregnant carnivora would determine whether or no the ova were lost in this way. I have examined one pregnant bitch and here the number of foetuses and corpora lutea were the same, namely five.

There is also the possibility that some of the ova may have escaped fertilization although this seems improbable.

It may be that, as indicated by the occurrence of atrophic foetuses, some of the fertilized ova do not develop very far but soon degenerate

[1] Rommel, *U.S. Dept. Agr., Bur. Anim. Indust.* Circ. No. 95, 1906.

[2] Surface, *Biometrika*, Vol. VI. 1908–9.

and traces of their implantation become lost. Todyo[1] has described a human ovum in which the atrophy began at a very early stage. Hill[2] found as many as 35 blastocysts in the uterus of the marsupial, Dasyurus, but of these 12 were abnormal. He states that cleavage abnormalities are quite frequently met with in Dasyurus.

The extent to which this factor comes into play in determining the fertility in the rabbit and pig will be seen by looking at column 6 of Tables II and III below.

TABLE II. *Rabbits.*

Number of rabbits	Date of copulation (about)	Days pregnant when killed (about)	Average no. of corpora lutea in ovaries per rabbit	Average no. of foetuses per rabbit	Average no. of ova of which no trace found per rabbit	No. of rabbits which contained ova of which no trace found	No. of rabbits containing atrophic foetuses	Average no. of atrophic foetuses per rabbit
5	Feb. 20	25	8·4	6·4	2·0	4	2	1·2
18	May 15	25	11·0	8·1	2·9	17	6	0·9
9	Oct. 15	9	9·1	7·6	1·4	3	1	0·1
6	,,	23	10·0	7·0	3·0	6	2	0·5

TABLE III. *Pigs.*

Sow	Days pregnant	No. of corpora lutea in ovaries	No. of foetuses in uterus	No. of atrophic foetuses	No. of ova of which no trace
IV	14 (?)	13	10	0	3
V	14 (?)	11	11	0	0
VI	30	24	21	0	3
VII	37	21	13	2	8
II	—	16	15	2	1
I	—	24	23	10	1
III	—	17	14	1	3
Average of 7 sows	—	18·0	15·3	2·1	2·7

On cutting open the pregnant uteri of pigs and rabbits one finds the majority of the foetuses about the same size and often a few very much smaller and of different degrees of development and in various stages of degeneration (see Figs. 1 and 2).

[1] Todyo, *Arch. f. Gynaek.* Bd. XCVIII. 1912.
[2] Hill, *Quar. Jour. Microp. Sci.* Vol. LVI. 1911.

This atrophy of some of the foetuses occurs also in other animals. Strahl and Henneberg[1] found atrophic foetuses frequently in rabbits and moles, less frequently in the hamster, and rarely in the ferret.

Many of the books on Veterinary Obstetrics describe mummified or "blighted" foetuses which are as a rule only observed on slaughter or when passed with the "cleansings" after a normal parturition; these have been observed both in the cow (de Bruin[2]), sheep and mare (Fleming[3]). The same thing also occurs in the human subject, or more rarely, the ovum perishing at an early stage degenerates into a "mole" (Galabin and Blacker[4]).

Tables II and III above show the extent to which these atrophic foetuses occur in the pig and rabbit.

Various stages of atrophy were seen in the animals examined. The foetus which weighed 9·5 grs in Sow I (Fig. 1) is probably in a condition which illustrates the first stages of the atrophy. The first sign is an oedematous swelling of the foetus while the membranes and placenta are tinged with blood.

The liquids then become absorbed and the foetus takes on a mummified condition looking very much as though it had been standing for some time in very strong alcohol. At first the foetus is a brick red colour but this eventually turns to a slaty grey; during this period also the amniotic fluid is largely absorbed and the foetal membranes atrophy. Often a brownish exudate is found in the cavity of the uterus in the vicinity of the atrophic foetuses. The placenta seems to be the last tissue affected and, in the rabbit, the atrophic foetus and its membranes may often be seen as a small mass situated on the summit of the still relatively large placenta (Fig. 2). In the atrophic foetuses of the pig the membranes seem to be loosely attached all round but eventually they dry and cling to one side of the uterus.

In animals bearing many young at birth this atrophy of the fertilized ovum during some stage of its devolopment is not very important economically since as a rule many more ova are shed than would furnish foetuses to occupy the teats of the mammary gland of the animal. Parker and Bullard[5] find however that in sows from the Corn belt of the Mississippi Valley the average number of nipples is 12 whereas the average litter is six. In animals producing only one or two young

[1] Strahl and Henneberg, *Anat. Anz.* Bd. xx. 1902.
[2] de Bruin, *Bovine Obstetrics* (trans.), London, 1901.
[3] Craig, *Fleming's Veterinary Obstetrics*, London, 1912.
[4] Galabin and Blacker, *The Practice of Midwifery*, London, 1910.
[5] Parker and Bullard, *Proc. Amer. Soc. of Arts and Sci.* Vol. XLIX. 1913.

at birth (mares and cows) however the matter becomes one of great importance, for the atrophy of the foetus here gives rise to complete sterility. It is significant also that in those animals which only produce one or two young at birth cases of complete sterility are most frequent. It is important therefore that the cause of this atrophy should be discovered.

The cause of atrophic foetuses.

On consideration of the case there seem to be three possible ways in which the atrophy might be caused—(*a*) Bacterial, (*b*) Nutritional, (*c*) Innate lack of vitality in the foetus.

(*a*) At first sight it appears that this atrophy might be due to some kind of bacterium somewhat similar to that of abortion, such as that recently described in sheep[1]; the foetuses however do not show any signs of bacterial action but become mummified. If this is a bacterial disease one would not expect to find, as is the case, healthy and diseased foetuses side by side in the uterus, the atrophic foetuses often being separated from one another by two quite healthy ones. From some atrophic foetuses contained in two rabbits cultures were made in nutrient agar tubes, both shake and slant, but only one colony appeared and this was of a common putrefactive type and probably of accidental occurrence. Sections have been cut from the atrophic foetuses of both the rabbit and pig; these when stained by the usual bacterial stains showed no bacteria. From this it would seem that the cause of the atrophy is not bacterial.

(*b*) Hill and O'Donoghue[2] found that in Dasyurus the food supply limited the fertility to a remarkable extent. Of 72 pregnant females examined 35 had more than 20 embryos while only three had less than six; six being the maximum number of young reared since there are only six nipples in the pouch. In several cases the foetuses in excess of this number were found in a state of atrophy in or near the pouch. Consequently it would seem possible that the cause of the atrophy in the Eutheria is of the same nature and depends on the size of the uterus. In support of this view it will be noticed that in the case of Sow I (Fig. 1) the atrophic foetuses are not all of the same size but appear to have dropped out one by one in a manner such as would occur in a struggle for nourishment with the increasing bulk of the

[1] Bd. of Agric. and Fish., *Rep. Com. on Epizootic Abortion*, Cd. 7157.

[2] Hill and O'Donoghue, *Quar. Jour. Microp. Sci.* Vol. LIX. 1913.

foetuses. If we accept this explanation of the atrophy we must look upon the small "piglings" (runts, darlings, etc.) which occur in almost every litter of pigs as those which would have been the next to drop out in the struggle for food in the uterus. There is much evidence that nutrition affects the size of the new born. Everard[1] found that the size and vigour was markedly affected by the nutrition of the dam during the period of gestation and Fleming states that with multiparous animals where the number of foetuses is smaller than usual the size is often increased to an abnormal degree.

To obtain evidence on the effect of nutrition some of the foetal membrane lengths in pigs were measured. It was thought that this would give some indication of the area available for obtaining nourishment and so give a measure of the nutrition of each foetus. Table IV below shows that on the whole the longer the foetal membranes the larger the foetus. This however can be looked at in another way: since the foetal membranes are outgrowths from the foetus, the greater its vitality the better will its membranes develop. In the case of Sow II although one foetus weighed only 1·5 grs. yet its membranes were not so small in proportion as the others.

TABLE IV.

Sow II															
Grms. weight of foetus	11·2	11·1	11·1	11·0	10·1	10·1	9·8	9·8	9·6	9·4	9·2	8·6	7·6	1·5	0
Inches length of foetal membranes	18·5	21	19	24	19	16	24	13·5	21	17	14·5	15	17	9·5	4·5
Sow III															
Grms. weight of foetus	44·3	43·5	43·2	43·0	42·4	39·9	39·2	39·0	38·9	37·4	37·1	36·5	28·8	—	—
Inches length of foetal membranes	29·5	28	25	28	29	26·5	25	21·5	22	24	21	16	18·5	—	—

A number of foetal rabbits and pigs have been weighed and the results (Table V) show that competitive nutrition has very little effect in determining the size of the foetus. Fourteen cases in rabbits and pigs have been examined in which the foetuses were unequally distributed in the horns of the uterus. In four cases the average foetal weight was smaller on the side which contained the fewer foetuses, in seven cases it was larger and in three cases the average foetal weight was the same on each side.

[1] Everard, *Science*, Vol. XXXVIII. No. 972, 1913.

TABLE V.

Rabbit	Total weight of foetuses	No. of foetuses	Average weight of foetus
V wild	1·04 4·08	1 4	1·04 1·02
γ_{24}	3·5 4·65	3 4	1·16 1·16
γ_{27}	6·93 0	5 0	1·39 0
III wild	1·48 2·97	1 2	1·48 1·48
I wild	5·98 12·23	2 4	2·99 3·06
γ_{23}	9·5 0	1 0	9·5 0
γ_{26}	28·5 39·0	3 5	9·5 7·8
γ_{2}	89·8 50·3	5 3	17·96 16·76
γ_{22}	97·5 92·5	4 4	24·4 23·12
IV wild	53·3 78·2	2 3	26·65 26·1
γ_{25}	107·8 128·2	4 5	26·95 25·64
γ_{17}	161·0 62·4	4 2	40·25 31·2
γ_{6}	130·5 174·0	3 4	43·5 43·5
Pigs			
VI	26·96 17·01	13 8	2·07 2·12
VII	33·88 28·35	6 5	5·64 5·67
II	69·0 61·1	7 6	9·86 10·2
I	113·0 110·0	6 6	18·83 18·33
III	281·8 232·4	7 6	40·26 38·73

If competition for nourishment causes the atrophy one would expect to find a greater percentage of atrophic foetuses on the side of the uterus where more eggs were embedded. The uterine horns of rabbits were grouped on the basis of the number of foetuses contained in them

and the percentage of atrophic foetuses in each group was calculated. The results given in Table VI show that the occurrence of atrophic foetuses is not associated with a large number of foetuses in one horn of the uterus. In one case where only two foetuses occurred in one horn of the uterus both were atrophic; in another there were eight foetuses in one horn but none showed signs of atrophy. The atrophy therefore is probably not due to competitive nutritional conditions.

Fraenkel[1] has shown however that the nutrition of the foetus is more especially under the control of the corpus luteum. He found that extirpation of both ovaries with the contained corpora lutea in rabbits was attended with atrophy of the foetus and absorption in utero, very similar to that which takes place under natural conditions; removal of the corpora lutea only by cautery had the same effect as extirpation of the ovaries.

TABLE VI. *Rabbits.*

Number of foetuses per uterine horn	Number of degenerate foetuses				Total number of foetuses	% of foetuses degenerating
	0	1	2	3		
8	1	—	—	—	8	0
7	3	1	—	—	28	3·57
6	3	2	2	1	48	18·75
5	6	2	1	1	50	14·0
4	5	0	1	—	24	8·33
3	7	1	—	—	24	4·17
2	6	0	1	—	14	14·3
1	3	—	—	—	3	0

The atrophy which takes place in nature however cannot be due to lack of luteal substance, since he found that the number of corpora lutea could be more than a half smaller than the number of foetuses and yet these mature normally. Strahl and Henneberg[2] caused a similar atrophy and absorption of the foetuses by cutting off the blood system, by ligaturing the maternal blood vessels or by pricking the foetal membranes and withdrawing the fluids. Since the pig has a *diffuse* placenta it seems unlikely that all the maternal blood supply could be cut off by a clot on the vessels and the atrophy caused by this means.

(c) It seems possible that the atrophy is due to the low vitality of the foetus. Marshall[3] concludes that a tendency towards sterility is often

[1] Fraenkel, *Arch. f. Gynaek.* Bd. LXVIII. 1903.
[2] Strahl and Henneberg, *Anat. Anz.* Bd. XX. 1902.
[3] Marshall, *The Physiology of Reproduction*, London, 1910.

associated with a constitutional loss of vigour. He performed an interesting experiment on an inbred Dandie Dinmont bitch. Equal quantities of semen collected from a Dandie Dinmont dog and a mongrel terrier dog were mixed together and injected into the bitch; 59 days afterwards four pups were born and these were all mongrels.

Heape's results on the crossing of Dorset and Hampshire sheep and Nathusius' experiments with pigs, showing that inbreeding may decrease fertility and an out-cross increase it, point to the conclusion that fertility is influenced by the male. Hyde[1] has found that if two strains of flies with low fertility are crossed, there is a sudden increase in the output; more eggs of each strain are fertilized by sperms from the other strain than when the eggs are fertilized by sperms from the same strain.

Possibly the atrophy of the embryo may be caused by reduced vitality due to something inherent in the foetus and thus may be often augmented by inbreeding. Bauer[2] has shown that in Antirrhinum the dying off of some of the seedlings is due to the uniting gametes lacking the factor for chlorophyll, the young plants perishing at an early stage because no assimilation can take place.

Cuénot[3] has suggested that the unexpected Mendelian ratio obtained when yellow mice are bred together is due to the fact that homozygous yellow zygotes are incapable of development. In 122 matings of yellow × yellow he obtained 419 young whereas 122 matings of yellow × some other colour gave 539 young. Blair Bell[4] states that a woman sterile with one husband is often fertile with another and this he attributes to incompatibility in the matter of fertilization. Morgan[5] obtained a mutant in the fly Drosophila, the females of which were absolutely infertile with males of the same kind but fertile with males of another strain; the males of this strain also were capable of fertilizing the females of other strains.

The atrophy of the foetus might also possibly be due to the condition of the male at the time of impregnation. Jennings[6] obtained the weakening effect in Paramoecium not only by inbreeding but by keeping under poor conditions. Centi[7] found that mutilation of the

[1] Hyde, quoted from Morgan's *Heredity and Sex*, p. 199.

[2] Bauer, *Zeits. f. indukt. Abstam. u. Vererbungslehre*, Bd. I. 1908 and Bd. III. 1910.

[3] Cuénot, *Arch. Zool. exp. et gén.*, T. IX. 1908–9.

[4] Blair Bell, *The Principles of Gynaecology*, London, 1910.

[5] Morgan, *Heredity and Sex*, New York, 1913.

[6] Jennings, *Jour. Exp. Zool.* Vol. XIV. 1913.

[7] Centi, *Arch. Ital. de Biol.* T. XLVIII. 1907.

cerebral cortex of mature fowls, both male and female, was attended by varying degrees of sterility and that many of the eggs gave abnormal germs; for example, embryos of the seventh and eighth day presented the same sort of development which exists normally at the second or third day.

Stockard and Craig[1] found that male guinea-pigs, which had been dosed heavily with alcohol, when mated with normal females produced only few young and these were weakly. Of 24 matings 14 had no offspring, five had stillborn litters and there were five living litters with 12 young, seven of which died shortly after birth.

Koebner[2] who sought the causes of the atrophy of the foetuses in rabbits gives the following table showing that they occur most frequently in May and August, but his numbers are far too small.

Table from Koebner.

Month	Number examined	Number of atrophic foetuses
Feb.	1	0
April	3	0
May	6	4 (in 1 rabbit)
August	3	10 (in 3 rabbits)
Oct.	2	1 (in 1 rabbit)
Nov.	1	0
Dec.	5	7 (in 4 rabbits)

The results given in Table I of this paper however do not show that there is any variation in the number of atrophic foetuses due to the time of year.

Koebner[2] also suggested that domestication may play a part in the occurrence of these foetuses. Darwin quotes many cases of animals which would not breed under confinement although copulation took place frequently. He mentions some cases however which did produce young but these were born dead and ill-formed.

A number of pregnant wild rabbits have been examined and Table VII below shows that in every case all the eggs shed developed into normal foetuses[3].

The data are insufficient to base any conclusion on but seem to suggest that the effect of domestication on the rabbit is to increase the number of

[1] Stockard and Craig, *Arch. f. Entwick.* Bd. xxxv. 1913.

[2] Koebner, *Arch. f. Gynaek.* Bd. xci. 1910.

[3] Further investigation has shown that degenerate foetuses do occur in wild rabbits but not to the same extent as in the domesticated breeds.

ova shed at each period but at the same time to reduce the proportion of these which develop.

TABLE VII.

Wild rabbit	I	II	III	IV	V	VI
Corpora lutea	2 + 1	2 + 4	1 + 2	2 + 3	1 + 3	1 + 3
Foetuses	2 + 1	2 + 4	1 + 2	2 + 3	1 + 4	1 + 3

SUMMARY.

After a consideration of the ways in which the fertility of domestic animals is controlled some of the factors which limit it have been investigated. Various circumstances control the number of ova shed at each heat period. Data are presented which show that the low fertility of young as compared with adult sows is due to the fact that not so many ova are shed at each period.

Counts have been made of the number of corpora lutea present in the ovaries and number of foetuses present in the uteri of pregnant rabbits and pigs. The results show that many more ova are shed at the heat period than young are produced at birth. Some ova possibly may be lost but many after fertilization atrophy at some period of their development and undergo absorption in utero.

While the occurrence of atrophic foetuses only causes reduced fertility in animals which have many young at birth yet their occurrence in animals producing only one young would give rise to sterility so that the problem of the cause of the atrophy becomes an important one.

Investigation points to the conclusion that the atrophy is not bacterial in origin since frequently healthy and atrophic foetuses lie side by side in the uterus. Moreover no bacteria could be found either in the foetus or foetal membranes.

Evidence is given to show that nutrition cannot be the cause of the atrophy although it may affect to a certain extent the size of the young.

No conclusion has yet been arrived at as to the cause of the atrophy, and the several possibilities suggested are still under investigation.

My thanks are due to Dr F. H. A. Marshall for the encouragement he has given me in this investigation.

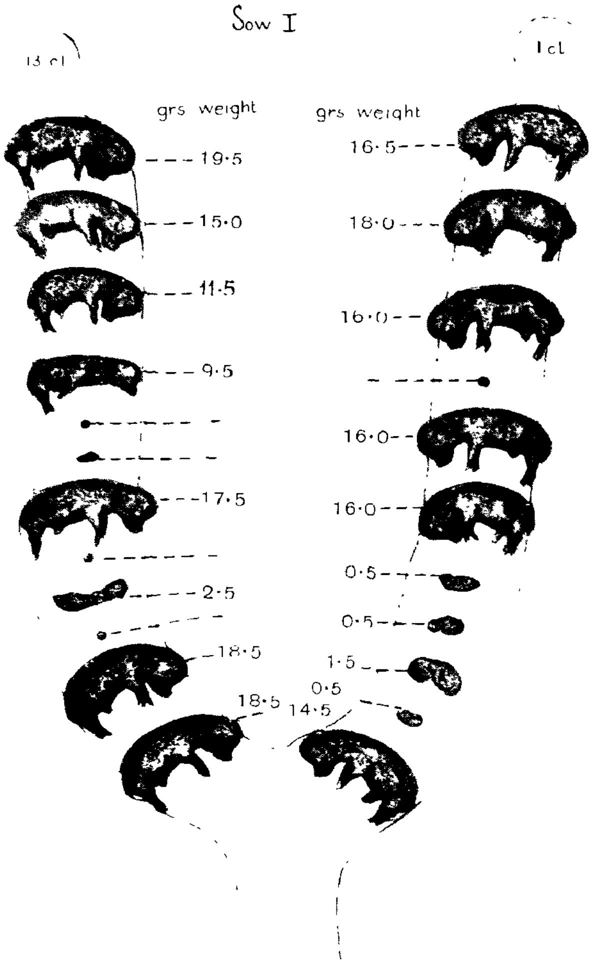

Fig. 1.

γ_{17}

The expenses of the investigation were largely defrayed by a grant made by the Development Commissioners through the Board of Agriculture and Fisheries to Dr F. H. A. Marshall.

EXPLANATION OF PLATE III.

Fig. 1. Photograph of the foetuses from Sow I showing their relative position in the uterus and the weight of each foetus. The positions of extremely small foetuses which consisted for the most part of membranes only are indicated by dots. The figures in the circles at the ends of the uterine cornua denote the number of corpora lutea present in the ovaries.

Fig. 2. The pregnant uterus of rabbit γ_{17}. The uterus has been slit open longitudinally, showing in the left horn three atrophic and two normal foetuses and in the right four normal and two atrophic foetuses. The atrcphic foetuses are seen as small light objects on the summit of the relatively large dark placenta. Six corpora lutea were present in each ovary.

THE SOIL SOLUTION AND THE MINERAL CONSTITUENTS OF THE SOIL.

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(From the Rothamsted Experimental Station.)

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INTRODUCTION.

It has long been the accepted theory that plants obtain the mineral constituents they require from the soil through the intermediary of a solution that is formed in the water with which the soil particles remain in contact. As, however, the amounts of phosphoric acid and potash revealed by analysis are always far in excess of the requirements of the crop, and as the variation in these quantities in no way determines the need or otherwise for a further supply of the constituents in the shape of fertilisers, Daubeny[1] suggested a distinction between dormant and available plant food in the soil, the latter being the more readily soluble compounds of phosphoric acid and potash, which in virtue of their solubility determine the amount of each constituent obtainable during the short season of a plant's growth. This point of view was revived by B. Dyer in 1894[2], and has been subjected to considerable examination, without, however, revealing any constant correspondence between the quantities of easily soluble plant food and the response of the soil to particular fertilisers.

A new aspect of the problem was set forth in 1903 by M. Whitney[3] and F. K. Cameron[4], who maintained that as all soils contain practically the same compounds of phosphoric acid and potash possessing a very

[1] *Phil. Trans.* 1845, p. 240.

[2] *Chem. Soc. Trans.* 1894, Vol. xcv. p. 115.

[3] Whitney and Cameron, Bull. 22, 1903, Bureau of Soils, U.S. Dept. of Agric.

[4] *Journ. Phys. Chem.* 1910, Vol. xiv. p. 320.

low solubility, the soil solution must become saturated with these constituents to the same low degree of concentration in all soils, irrespective of the actual amounts of phosphoric acid and potash there present.

"From the results of other investigations described and the figures given in the preceding tables the conclusion seems inevitable that all our principal soil types—in fact practically all cultivable soils—contain naturally a nutrient solution which varies within comparatively narrow limits with regard either to composition or concentration, and which is usually sufficient for plant growth. Apparently, therefore, all these soils are amply supplied with the necessary plant foods, and these plant foods are not in themselves a matter of such paramount importance to the agriculturist[1]."

"This water is moving over the soil particles in films and with slowness. It is long in contact with successive fragments of any particular mineral and all the different minerals making up the soil. Consequently it tends towards a saturated solution with respect to the mineral mass, and it follows that if every soil contains all the common rock-forming minerals, every soil should give the same saturated solution, barring the presence of disturbing factors[2]."

The authors thus postulate a soil solution of approximate constant composition, and further adduce evidence that this solution, though of great dilution, is capable of satisfying the requirements of the plant, the growth of which (as they maintain) is independent of the concentration of the solution within very wide limits.

It would follow as a corollary that the addition of a soluble fertiliser has no permanent effect in raising the concentration of the soil solution, because it immediately reacts with the comparatively large mass of minerals present (phosphoric acid with basic compounds of calcium, magnesium, iron, and aluminium; potash with the zoolitic silicates) to form compounds of the same kind as those naturally present in the soil, so that the original equilibrium between the soil solution and these latter compounds is quickly restored with but slight disturbance. Thus they reach the general conclusion that the quantity of mineral plant food in the soil is without significance in the nutrition of the plant, that the observed differences in the fertility of soils are in the main to be attributed to the varying capacity of soils to maintain a supply of water to the plant, and that the response of the crop to particular

[1] Whitney and Cameron, *loc. cit.* p. 46.
[2] Cameron, *loc. cit.* p. 351.

fertilisers may be set down, not to an additional supply of plant food, but to a precipitating or inhibiting action of the fertiliser upon specific toxins excreted by the plant and possessing a depressing effect upon the same kind of plant when grown again in the same soil.

Little as this view would seem to square with our experience of the effects of phosphatic and potassic fertilisers on particular soils, the theory of a soil solution of constant composition must be valid if the conditions existing in the soil are such as postulated by Whitney and Cameron. The supposition that the plant's roots exert a solvent action upon the solid particles of the soil has been generally abandoned, for the etching effects observed in the classic experiment of Sachs[1] can be explained by the carbon dioxide excreted by the roots. It is true that the soil solvent is not pure water, but a dilute solution of this carbon dioxide, yet as the carbon dioxide tension of the air in the soil varies within small limits, this solution would equally become saturated with the phosphoric acid and potash, and the authors' argument would not be invalidated.

Before the theory can be accepted two points appear to require examination. In the first place the soil solution may not be of constant concentration, because the soil minerals may not be so similar as is supposed, especially after the application of fertilisers. Whitney and Cameron's figures on the point are not convincing and require confirmation. But even if the soil solution does vary in concentration, Whitney and Cameron's point of view would still hold, unless it is further shown that the plant's growth varies in response to the concentration, irrespective of the total supply of plant food.

Secondly, if a soil solution of constant composition is granted, the maintenance of this solution as the nutrients are extracted from it by the plant's growth may become a factor of importance, determined by the amount of the constituent present in the solid state. The soil solution exists in thin films coating the soil particles; the roots are only in contact with these films over a limited area; should they deplete the films there may be such a lag in the solution of more solid and its travel to the roots as will cause an appreciable difference in the rate at which the plant is supplied from a soil containing little plant food as compared with one possessing a more abundant stock.

The following investigation was set on foot in order to test these points of view and to elucidate the nature and function of the soil solution in the nutrition of the plant.

[1] *Text-Book of Botany*, English ed., 1875, p. 625.

I.—GROWTH OF PLANTS IN SOIL SOLUTIONS.
(A. D. H. and W. E. B.)

For the purposes of the investigation it was necessary to compare the growth of plants in soil solutions alone, so that neither the direct action of the plants on the soil nor the rate of renewal of the solution from the soil could be factors in the result. The method of water cultures adopted is subject to many disturbances and errors, which were as far as possible minimised as follows:—

1. Each plant grew in its own bottle, holding about 600 c.c. of solution.

2. A "pure line" of seed was chosen, and seeds were selected falling within certain limits of weight.

3. Each unit of comparison in the experiment consisted of 10 plants; the figures given represent the mean dry weight of the plants forming the unit.

4. The growth took place in a greenhouse, beginning in early spring; in the summer growth is unsatisfactory, and the plants are liable to fungoid disease.

Because of the difficulty of obtaining any considerable volume of the soil solution as it exists in the soil, one was artificially made by slowly and thoroughly working up such a quantity of the soil in the moist condition in which it came from the field as would produce a mixture containing 20 kgrm. of dry soil and 35 kgrm. of water. On the following day, after settlement, the supernatant liquid was syphoned off and filtered through a Berkefeld filter. In the later experiments an asbestos wool filter was substituted instead, as the Berkefeld proved very slow in action. The contents of the bottles were renewed at fortnightly intervals, fresh solutions being made up for the purpose from newly dug soil. Owing to mechanical difficulties it was impossible to renew in each day more than the solutions constituting one unit, but the units were treated in rotation, and each had the same total period of growth. This renewal of the solutions ensured that the growing plants should never be suffering from lack of nutrient through a depletion of the solution.

The soils were selected from certain of the plots of the permanent wheat and barley fields at Rothamsted, of which the treatment with fertilisers and the crop-producing powers for 60 years are now on record. The plots selected in the two fields had not received fertilisers identical in all respects, but the treatment had been very similar as

regards mineral fertilisers. The supply of nitrogen admittedly does not enter into the problem, because this element only reaches the plant after conversion by bacterial action of its insoluble compounds into nitrates and ammonia, compounds which pass wholly into solution. In order that the supply of available nitrogen should not be a factor in the results, to each solution sodium nitrate, at the rate of 0·25 grm. per litre, was added.

Table I shows the manuring and the average crop produced on the selected soils during the last 10 years, 1902–11.

TABLE I. *Yield of Wheat and Barley. Rothamsted,* 1902–11.

Character of manuring	Wheat—Broadbalk				Barley—Hoos			
	Plot	Yield per acre			Plot	Yield per acre		
		Grain	Straw	Total produce		Grain	Straw	Total produce
		bushels	cwt.	lb.		bushels	cwt.	lb.
Unmanured continuously ...	3	10·9	9·6	1801	1, o	9·3	6·2	1276
N only, no P_2O_5, no K_2O ...	10	18·4	15·0	3256	—	—	—	—
$N+P_2O_5$	11	19·2	21·8	3778	2A	29·7	19·3	3972
$N+K_2O$........................	—	—	—	—	3A	20·3	15·6	2985
Complete artificial fertiliser	7	31·0	35·5	6015	4A	38·4	25·3	5087
Dung every year	2	35·1	40·8	6925	7/2	44·2	31·6	6184

Wheat was grown in both sets of solutions, those from the soil of the wheat and the barley field; similarly, barley was grown in the solutions from both the barley and wheat field. Growth was good from the outset, and was continued from March to June, each unit growing for a period of eight weeks, after which the roots were washed, and root and shoot were dried and weighed separately. Differences in the rate of development were manifest in the first fortnight, and persisted to the end; it was noticed that the plants growing in the solutions from the dunged soil appeared to have a greater superiority in vigour, especially in their broad green leaves, than was indicated by their final weights. The mean results are set out in Table II.

The important point brought out by these results is that the growth in the solutions of the various soils is not identical, but shows differences which are very parallel to the differences in the growth of the crop on the soils themselves in the field. The diagram (Text-fig. 1) shows a comparison between the yields from the soil solutions and the yield of the

crops in the field from the corresponding soils (10 years' average, total produce, 1902–11). The same sequence is preserved in the two cases, but the differences are more pronounced in the solutions, except as regards the unmanured plot, which in the solution receives the nitrogen that is lacking in the field. Further, the growth in the solutions

TABLE II. *Growth of Wheat and Barley in Solutions of Rothamsted soils. Series I, 1911.—Mean dry weight in grammes.*

Soil	Wheat				Barley			
	Shoot	Root	Total	Ratio Shoot/Root	Shoot	Root	Total	Ratio Shoot/Root
Wheat, Plot 3	0·170	0·135	0·305	1·26	0·212	0·105	0·317	2·02
,, ,, 10	0·157	0·127	0·284	1·24	0·171	0·101	0·272	1·69
,, ,, 11	0·598	0·260	0·858	2·30	0·660	0·175	0·835	3·77
,, ,, 7	0·923	0·448	1·371	2·06	1·302	0·442	1·744	2·95
,, ,, 2	1·137	0·425	1·562	2·68	1·249	0·377	1·626	3·31
Barley, Plot 1, o	0·240	0·169	0·409	1·42	0·264	0·138	0·402	1·91
,, ,, 2A	0·476	0·199	0·675	2·39	0·611	0·137	0·747	4·46
,, ,, 3A	0·208	0·201	0·409	1·03	0·275	0·119	0·394	2·31
,, ,, 4A	1·203	0·627	1·830	1·92	1·600	0·477	2·077	3·35
,, ,, 7/2	1·195	0·511	1·706	2·34	1·364	0·486	1·850	2·81

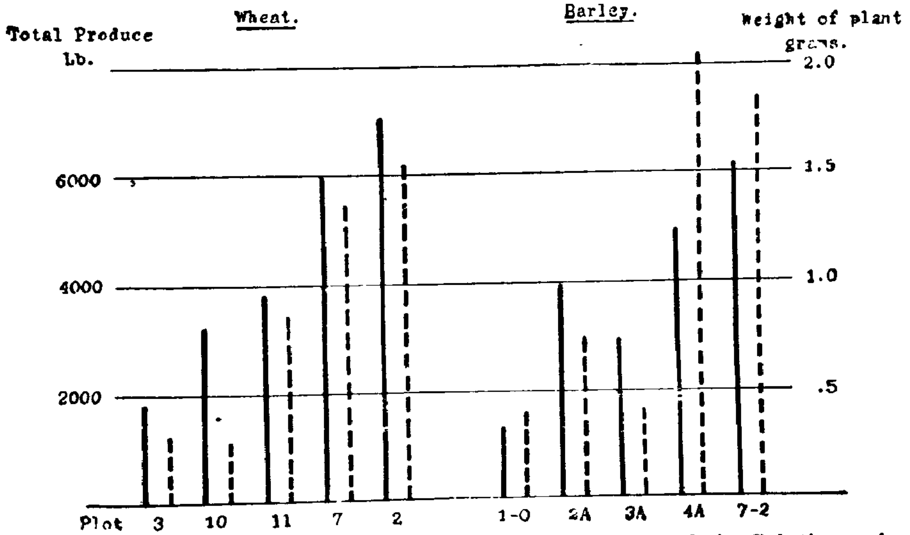


Fig. 1. Comparison of Crop on Rothamsted Plots with Growth in Solutions of same Soils. Solid line = crop, total produce 1902-11. Broken line = dry weight of plant in soil solution.

is such as would be anticipated from the composition of the solutions, which corresponds, though in a very approximate fashion, to the past manurial history of the soils, and to their composition as revealed by analysis. Large volumes of the solution from each soil were evaporated, and analyses were carried out by the standard methods, with the results set out in Table III. For comparison, the amounts of phosphoric acid

TABLE III. *Composition of Soil Solutions and Soils—Rothamsted.*

Field and Plot	Phosphoric acid: Soil solution, parts per million	Phosphoric acid: Soil, Total	Phosphoric acid: Soil, Citric acid soluble	Manure annual supply, lb. per acre	Potash: Soil solution	Potash: Soil, Total	Potash: Soil, Citric acid soluble	Manure annual supply, lb. per acre
		per cent.	per cent.			per cent.	per cent.	
Wheat, Plot 3	0·656	0·114	0·0078	0	3·64	0·220	0·0032	0
,, ,, 10	0·881	0·123	0·0074	0	3·55	0·240	0·0032	0
,, ,, 11	3·839	0·197	0·0405	60	3·88	0·197	0·0032	0
,, ,, 7	3·938	0·195	0·0547	60	26·22	0·262	0·0232	100
,, ,, 2	4·838	0·215	0·0560	46	29·85	0·285	0·0384	60
Barley, Plot 1, o	0·525	0·099	0·0055	0	3·40	0·183	0·0036	0
,, ,, 2A	3·900	0·173	0·0425	60	3·88	0·248	0·0023	0
,, ,, 3A	0·808	0·102	0·0081	0	30·33	0·257	0·0407	100
,, ,, 4A	4·025	0·182	0·0500	60	24·03	0·326	0·0298	100
,, ,, 7/2	4·463	0·176	0·0447	46	26·45	0·167	0·0321	60

and potash in the soil, both soluble in strong hydrochloric acid and in a 1 per cent. solution of citric acid, are appended, as well as the amount annually supplied to the soil in the manure. It will be seen that, where there had been no phosphoric acid supplied, the amount in the soil solution varies from 0·525 to 0·881 parts per million, but rises to about four parts per million in the solution of the soil from plots receiving phosphoric acid annually. Similarly, the potash in the solutions of soils from plots not receiving potash approximates to 3·5 parts per million, but rises to between 24 and 20 parts per million in the solutions from plots receiving potash annually.

The relative composition of the soil solutions is also similar to that of the soils, as judged either by the "total" or the "available" plant food they contain.

Thus the growth made by plants in the soil solutions is such as would be expected from the composition of the solutions, and the past

history and the present composition of the soils from which the solutions were made.

The experiments were resumed in the following year, and, as the results given by the wheat and barley soils had been so similar, they were on this occasion confined to solutions made from the barley soils only. In order to examine further the conclusion that no other factor entered into the effects produced by the soil solutions than the amount of plant food they contained, comparisons were made between (1) a culture solution made up in the laboratory to the same concentration in essential nutrients as the solutions from the completely manured soils, (2) the soil solutions, (3) the soil solutions from the partially manured plots, with their essential deficiencies repaired by the addition of phosphoric acid or potash, or both. A further artificial solution (4) was made up to a much higher concentration, to one in common use in the laboratory; lastly (5), the salts in this artificial solution were added to the soil solutions. Thus, for each plot, the following comparisons were obtained:—

Nature of solution	Nutrients, parts per million: From soil P_2O_5	From soil K_2O	From added salts P_2O_5	From added salts K_2O	
1. Artificial culture solution ...	Nil	Nil	4·7	26·7	Low concentration
2. Soil solution ...	0·6 to 4·7	3·4 to 26·7	Nil	Nil	Varies with plot
3. Soil solution with added salts	0·6 to 4·7	3·4 to 26·7	4·5	26·5	Varies with plot; additions as required
4. Artificial culture solution ...	Nil	Nil	303·5	312·4	High concentration
5. Soil solution with added salts	As (2)	As (2)	303·5	312·4	,, ,,

As before, in all cases sodium nitrate was added at the rate of 0·25 grm. per litre, or 41 parts nitrogen per million. Barley plants were grown for seven weeks, from March to April, with the results set out in Table IV.

From these results the following conclusions may be drawn. The artificial culture solution, which was calculated to be approximately equivalent to the soil solutions yielded by the completely manured plots 4A and 7/2, yielded plants whose weight (0·763) was distinctly lower, but of the same order, as those grown in the soil solutions from the completely manured plots (0·963 and 1·465). The artificial culture

solution of high concentration yielded heavier plants (0·943), approaching those obtained in the solutions from the completely manured soils, though still below the maximum. The soil solutions from the unmanured (1, o) and imperfectly manured plots (2A) yielded plants of a much lower order of magnitude (0·216 and 0·486). The addition of the missing nutrients to the solutions from the imperfectly manured soils produced growth approaching the maximum (1·214 and 1·154); when the nutrients were added to set up the higher concentration, the growth produced was equal to that obtained from the artificial culture solution of the same concentration (0·974 and 0·925 against 0·943), though still below the maximum.

TABLE IV. *Growth of Barley in Solutions from Hoos Barley Plots. Series II*, 1912.

Set	Nature of solution	Shoot	Root	Total	Ratio Shoot/Root
1	Artificial culture solution, low concentration	0·514	0·249	0·763	2·06
4	,, ,, high ,,	0·652	0·291	0·943	2·24
2	Soil solution, Plot 1, o, unmanured	0·116	0·100	0·216	1·16
3	,, ,, + added salts, low concentration	0·865	0·349	1·214	2·48
5	,, ,, ,, high ,,	0·677	0·297	0·974	2·28
2	Soil solution, Plot 2A, lacking potash	0·353	0·133	0·486	2·65
3	,, ,, + added salts, low concentration	0·795	0·359	1·154	2·22
5	,, ,, ,, high ,,	0·619	0·306	0·925	2·02
2	Soil solution, Plot 4A, complete fertiliser	0·685	0·278	0·963	2·47
5	,, ,, + added salts, H. C.	0·926	0·423	1·349	2·19
2	Soil solution, Plot 7/2, dunged	1·069	0·396	1·465	2·70
5	,, ,, + added salts, H. C.	0·814	0·472	1·286	1·73

These results amply confirm the conclusion drawn from the previous set of experiments—that the growth made by plants in the soil solutions is in the main determined by the amount of plant food they contain. One other point was suggested by the results, that the soil solutions, particularly those from the soil of the dunged plot, were better media for growth than the artificial culture solutions of equivalent concentration, possibly owing to the presence of soluble nitrogen compounds specially valuable to the plant in the earlier stages of growth. On the other hand, it is unsafe to lay much stress on such differences in weight as are exhibited in the growth of the plants in the solutions regarded as complete (0·943, 1·214, 0·974, 1·154, 0·925, 0·963, 1·349, 1·465, 1·286).

In order to check the conclusions still further, a third series of experiments were made. A solution containing per million 4·5 of

phosphoric acid and 26·5 of potash was taken as a standard, this being the approximate composition of the solutions of soils from the completely manured plots of the barley fields 4A and 7/2. To the solutions from the imperfectly manured plots (1, O, 2A, 3A), phosphoric acid and potash were added, (1) in amounts required to bring the proportion of these constituents up to the standard, allowance being made for the small amount already present that had been derived from the soil, (2) in amounts equal to the standard, over and above those which the soil had supplied. A set was also included to which no addition of sodium nitrate was made; to the rest, nitrates equivalent to 40 parts N per million were added. In Series II there was a slight imperfection in the comparison, because the added nutrients were equal to those contained in the solutions from the completely manured plots, thus, in the solutions from the imperfectly manured plots to which nutrients were added, there was a small surplus of nutrient derived from the soil.

Barley was again grown, and the growth was prolonged for four weeks only, from June to July. The cool, sunless season of 1912 enabled trustworthy experiments to be made at so late a period. The results obtained are set out in Table V, the dry weight of the whole plant only being given.

The results of this series are in strict conformity with those of the preceding series. It is noteworthy that the addition of nitrogen to the soil solutions produced no increase in the plant, indicating that the soils themselves had yielded more than enough nitrate for the needs of the plant, the growth of which had been limited by the amount of phosphoric acid and potash present in the solution. The evidence was slight for the presence in the soil solutions, even in those from the dunged plot, of other substances favourable to growth.

In order to check the conclusions still further the set of experiments in this series was duplicated with peas instead of barley (lower part of Table V).

Peas do not form so sensitive a culture plant as barley because the growth is maintained for a long time on the food store in the seed itself, but when allowance is made for this fact and the somewhat greater margin of error, the results obtained with peas were in complete accord with those yielded by barley.

The growth in the soil solutions being such as would be expected from the composition of the solutions and comparable with the growth of crops on the soils themselves, it is interesting to compare the composition of these artificially made solutions with that of the natural soil

TABLE V. *Barley and Peas in Solutions from Hoos Field Barley Soils. Series III*, 1912.

	Artificial culture solution	Plot 1, o, unmanured	Plot 2A, lacking potash	Plot 3A, lacking phosphoric acid	Plot 4A, complete manure	Plot 7/2, dunged
	Parts per million					
Phosphoric acid in original solution	4·5	0·525	3·90	0·808	4·025	4·463
Potash in original solution ...	26·5	3·40	3·88	30·33	24·03	26·45
Nature of solution	Dry weight of plant					
Barley—						
Solution only	0·319	0·149	0·213	0·167	0·303	0·403
,, + N	0·292	0·149	0·226	0·184	0·346	0·404
,, + N, P_2O_5 and K_2O to standard	—	0·420	0·395	0·323		
,, + N + standard P_2O_5 and K_2O	0·366	0·435	0·400	0·364	0·358	0·438
Peas—						
Solution only	1·731	1·082	1·184	1·192	1·449	1·630
,, + N	1·524	1·157	1·404	1·335	1·720	1·743
,, + N, P_2O_5 and K_2O to standard	—	2·299	1·800	1·961		
,, + N + standard P_2O_5 and K_2O	1·769	2·553	2·493	2·136	2·157	2·182

solution as far as it can be ascertained. The drainage from the Rothamsted wheat plots is collected separately by a tile drain running below each plot, and on various occasions when the drains ran during the progress of these experiments samples were taken and analysed as below.

It is not to be expected that the drainage water will represent the soil solution. When rain falls on the surface one of its effects will be to push downward the soil water already present until a saturated layer forms (*i.e.* a layer containing more water than the soil particles can hold by surface tension) and this layer travels downward until it reaches the drain, which then begins to run. In the drainage water there will always, however, be a certain dilution due to rainwater which has travelled more rapidly down cracks, worm tracks, and other large cavities, and this dilution will increase with the time that elapses after the drain begins to run. The analyses in Table VI show that the drainage waters were always more dilute than the solutions made from

the corresponding soils, but their content in phosphoric acid and potash is of the same order of magnitude and follows the same sequence as the soil solutions. Thus our conclusion is strengthened that the soil solutions experimented upon do represent the natural solution existing in the soil *in situ*.

TABLE VI. *Phosphoric Acid and Potash found in Soil Solutions and Drainage Waters at various Dates. Parts per million.*

	Soil solution	Drainage water 10/1/11*	Drainage water 2/4/11†	Drainage water 14/5/11†
	Phosphoric acid			
Plot 3. Unmanured	0·656	0·236	0·520	0·622
,, 10. Ammonium salts only	0·881	0·395	0·541	0·502
,, 11. ,, + phosphoric acid	3·839	0·642	0·608	0·961
,, ‡13. ,, ,, + potash	3·938	0·620	0·780	0·918
	Potash			
Plot 3	3·64	2·04	5·29	7·15
,, 10	3·55	1·07	3·88	4·87
,, 11	3·88	1·89	10·49	3·55
,, 13	26·22	2·94	11·77	13·55

* The drains had been running two days earlier.

† The drains had not run during the preceding month.

‡ Plot 13, the drainage water from which was analysed, receives the same manurial treatment as Plot 7, the soil of which was used for the soil solution analysed.

We may now consider how far these results bear on the theory that crops leave behind in the soil specific toxins which depress the growth of succeeding crops of the same kind. In Series I wheat and barley yielded almost exactly the same weight of plant, whether they grow in solutions from the wheat or the barley soils (Table I). As a rule the wheat plants were a little heavier when grown in the solutions from the barley soils than when grown in solutions from the corresponding wheat soils (3 compares with 1, O, 11 with 2A, 7 with 4A, 2 with 7/2), but the barley plants were similarly heavier in the solutions from the barley soils. The ratio of root to shoot is very close in the two sets. Again, wheat and barley grown in the same solution yield weights agreeing within the range of error of such experiments. These facts

alone would dismiss the hypothesis that the wheat soils contain any soluble toxin injurious to wheat but not to barley, and *vice versâ*, notwithstanding the 60 years' repeated growth of these crops on the same soils. In Series II the demonstration was pushed a stage further by including in the comparison an artificial culture solution made from pure salts and containing phosphoric acid and potash in the same proportions as the solutions from the completely manured plots. Another set of the soil solutions was boiled before use, since boiling had been reputed to destroy the toxin and would at any rate kill off any bacteria that might be factors in the result. Lastly in another set the solutions were evaporated, the residue ignited and dissolved afresh in a minimum quantity of hydrochloric acid, then diluted to the original volume. The results obtained are set out in Table VII.

TABLE VII. *Growth of Barley in Solutions from Hoos Field Barley Plots. Weight of Plant in grammes. Series II*, 1912.

Set	Treatment of solution	Artificial culture solution	Plot 1, o, unmanured	Plot 2A, lacking potash	Plot 4A, complete manure	Plot 7/2, dunged
2	Soil solution, unboiled	0·763	0·216	0·486	0·963	1·465
6	,, boiled	—	0·212	0·601	0·956	1·253
3	,, with added nutrients, unboiled	—	1·214	1·154		
7	,, with added nutrients, boiled	—	1·385	1·022		
8	,, residue ignited and re-dissolved	—	0·203	0·396	0·660	0·604

In this series boiling was without effect, whether the solutions contained added nutrients or not; the residue left on evaporation, after ignition and re-solution, gave generally lower results, in some cases to a marked degree. The soil solutions from completely manured plots gave higher yields than the artificial solutions of corresponding strength.

In order to ascertain whether the results were limited in any way by the nature of the plant (it might be objected as regards Series I that barley and wheat are so closely akin as to excrete the same toxin) the experiments in Series II were repeated with sunflowers, white lupins, and buckwheat, with the results set out in Table VIII.

These plants are far from being so suitable for experiment as barley, and the results are somewhat erratic (*e.g.* white lupins gave almost their maximum yield in the solution from the unmanured plot, indicating that growth had been mainly sustained on the original food

TABLE VIII. *Growth of Various Plants in Solutions from Hoos Field Barley Soils. Weight of Plant in grammes,* 1912.

Plant	Treatment of solution	Artificial culture solution	Plot 1, o, un-manured	Plot 2A, lacking potash	Plot 4A, complete manure	Plot dun
uckwheat	Soil solution, unboiled	—	0·178	0·300	0·708	0·9
,,	,, boiled	—	0·265	0·505	1·145	0·9
,,	,, unboiled, added nutrients	—	0·668	0·986		
,,	,, boiled, added nutrients	—	0·634	0·729		
hite lupins	Soil solution, unboiled	—	1·691	0·845	1·248	1·4
,,	,, boiled	—	1·685	0·825	1·724	1·(
,,	,, unboiled, added nutrients	—	1·086	1·170		
,,	,, boiled, added nutrients	—	1·898	1·282		
unflowers	Soil solution, unboiled	—	0·335	0·314	1·075	1·8
,,	,, boiled	—	0·338	0·651	—	0·7
,,	,, unboiled, added nutrients	—	1·366	1·205		
,,	,, boiled, added nutrients	—	Failed	1·176		

store in the seed), but they in no way indicate the presence of a toxin in the soil solutions which depresses the growth of barley but *ex hypothesi* is without effect on plants of another order. Finally in Series III (Table V) both barley and peas grew as freely in the soil solutions from the completely manured plots and in the solutions from the incompletely manured plots after repair of the deficiency by adding salts, as in the artificial solutions made up with pure salts. Indeed the superiority, though hardly large enough to be significant, lay with the plants grown in the soil solutions. Thus the experiment yielded no evidence of the existence in soils on which a particular plant had been growing for 60 years and upwards, of a soluble "toxin" having a depressing effect upon the growth of that plant.

II.—THE RELATION BETWEEN THE GROWTH OF THE PLANT AND THE CONCENTRATION OF THE NUTRITIVE SOLUTION.

(A. D. H. and L. M. U.)

As the second stage in the investigation it was necessary to ascertain if the concentration of the soil solution is a factor in the rate of growth of the plant. It has been generally assumed[1] that, within very wide limits, the plant will be indifferent to the concentration of the soil

[1] Binner and Lucanus, *Landw. Versuchs.-Stat.* 1866, Vol. VIII. p. 128; Cameron, *loc. cit.*, p. 403.

solution, provided that the total amount of nutrients available, in this case of phosphoric acid and potash, is adequate.

A standard solution was made up containing per litre 0·5 grm. each of potassium di-hydrogen phosphate, magnesium and calcium sulphates, and sodium chloride, 1·0 grm. potassium nitrate, and 0·04 grm. ferric chloride, equivalent to N 138, P_2O_5 261, and K_2O 743 parts per million. Barley was grown in bottles containing 600 c.c. of the above solution, at full strength, and diluted to 1/5, 1/10, and 1/20 respectively, the trials being made in duplicate only. Growth proceeded for eight weeks from March 10 to May 8, with the result set out in Table IX (see also Pl. IV, fig. 2[1]).

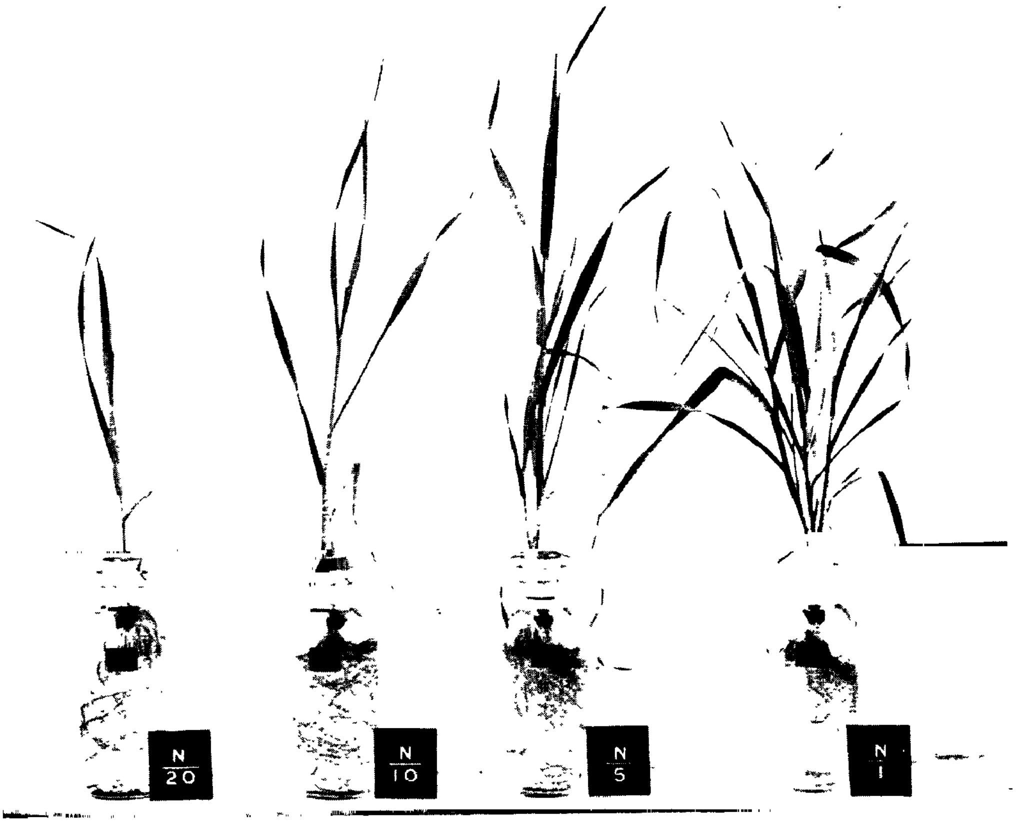
TABLE IX. *Growth of Barley in Nutritive Solutions of varying Concentration. March 10—May 8, 1911.*

Concentration of solution	Dry weight of plant in grammes			
	Shoot	Root	Total	Ratio $\frac{\text{Shoot}}{\text{Root}}$
1	1·323	0·332	1·655	4·0
	1·605	0·470	2·075	3·4
1/5	0·977	0·268	1·245	3·7
	1·087	0·405	1·492	2·5
1/10	0·742	0·288	1·030	2·6
	0·690	0·253	0·943	2·7
1/20	0·462	0·219	0·681	2·1
	0·369	0·168	0·537	2·2

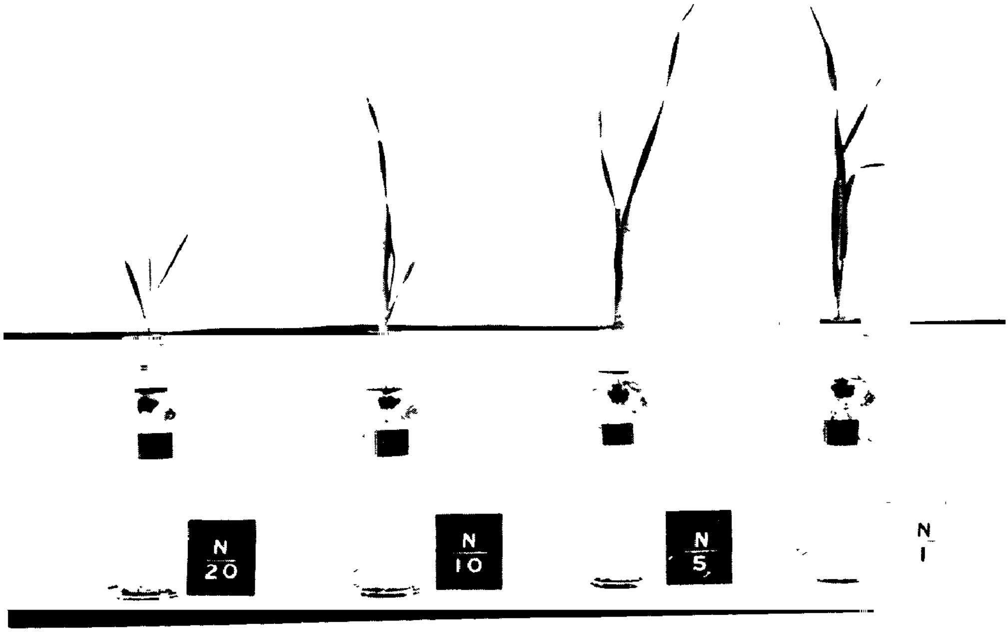
From the very outset the growth in the various solutions proceeded in the order of their concentration, so that the final weights may be taken to represent the rates of growth throughout and not an ultimate condition brought about by the exhaustion of the food supply, though the more dilute solutions were at the finish depleted of the nitrogen they originally contained. In order to obviate the effects of this depletion of the solution, in the next set of experiments the solutions were renewed weekly, the other conditions remaining as before, with the results set out in Table X.

This series, grown late in the season, was less satisfactory, but the results are confirmatory of those obtained before, and some unused

[1] Thanks are due to the Manchester Literary and Philosophical Society for the loan of the blocks for Figs. 2, 3, 4 and 6.



After 8 weeks.



After 4 weeks.

nitrate remained in all the solutions, except after the last two weeks' growth in the solutions of greatest dilution.

TABLE X. *Growth of Barley in Nutritive Solutions of varying Concentration renewed weekly. May 18—June 20, 1911.*

Concentration of solution	Dry weight of plant in grammes			
	Shoot	Root	Total	Mean
1	0·187 0·450	0·078 0·124	0·265 0·574	0·420
1/5	0·235 0·095	0·084 0·074	0·319 0·169	0·244
1/10	0·048 0·047	0·038 0·084	0·086 0·131	0·108
1/20	0·034 0·047	0·028 0·027	0·062 0·074	0·068

A new series was now arranged in which the plants were grown in coarse sand contained in vertical glass cylinders through which the nutritive solution slowly percolated. The cylinders contained 800 grm. of coarse sand mixed with 4·25 grm. calcium hydrogen phosphate (the potassium phosphate was withdrawn from the nutritive solution) and 100 c.c. of the nutritive solution daily was allowed to drip very slowly

TABLE XI. *Growth of Barley in Nutritive Solutions of varying Concentrations percolating through Sand.*

Concentration of solution	Shoot	Root	Total	Ratio $\frac{\text{Shoot}}{\text{Root}}$
1	2·969 2·393	0·769 0·787	3·738 3·180	3·5
1/5	1·218 1·698	0·304 0·555	1·522 2·253	3·4
1/10	1·148 0·837	0·690 0·221	1·838 1·058	2·2
1/20	0·488 0·603	0·280 0·308	0·768 1·011	1·8

on the sand, percolate through it and escape. Growth was continued for two months, March 26 to May 21, with results set out in Table XI (see also Pl. V, fig. 3).

The solutions escaping from the sand were regularly tested and found to contain nitrate, except in the last weeks of growth with the more dilute solutions.

Though late in the season for satisfactory work with water cultures two more experiments were made in 1912, in which barley and lupins were employed as test plants and 500 c.c. of solution were dropped through daily, with results set out in Table XII.

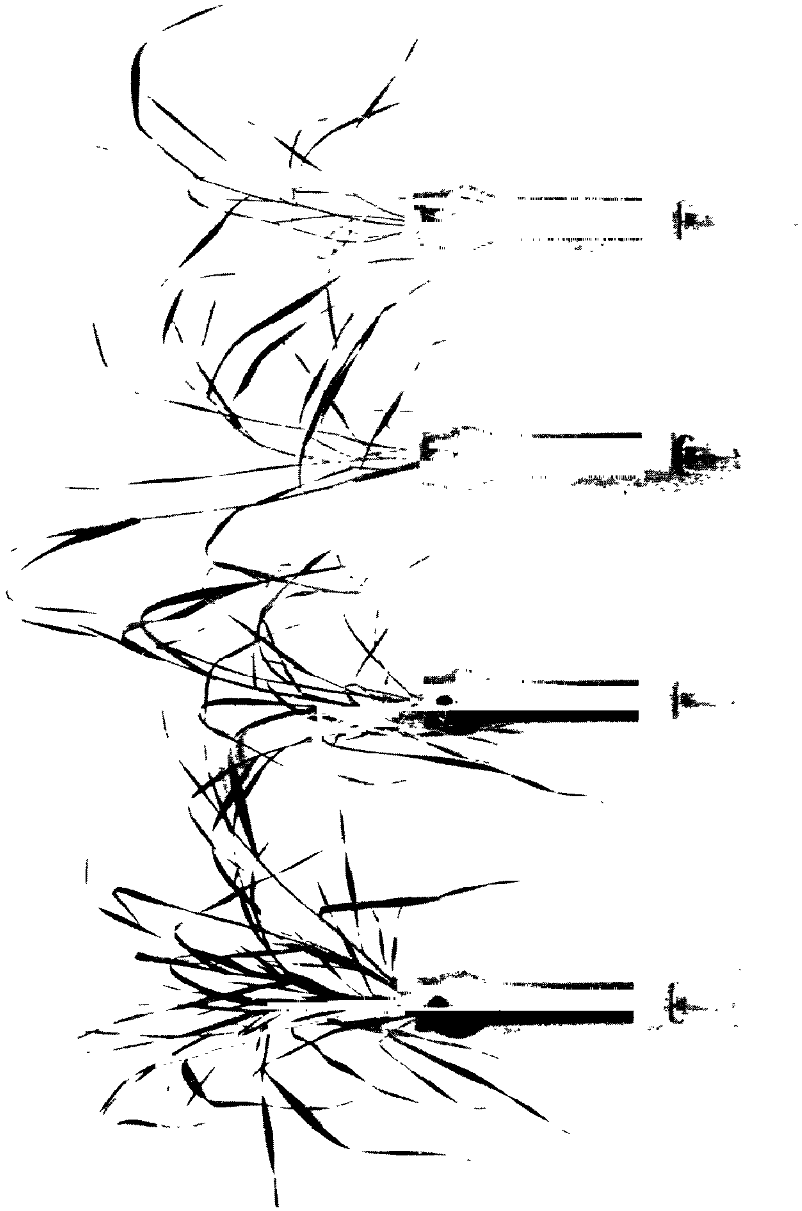
TABLE XII. *Growth of Barley and White Lupins in Nutritive Solutions of varying Concentration percolating through Sand.*

Concentration of solution	Dry weight of plant in grammes					
	Barley, 11/6/12 to 17/7/12			White lupins, 25/7/12 to 26/8/12		
	Shoot	Root	Total	Shoot	Root	Total
1	0·614	0·136	0·750	0·92	0·403	1·323
	1·128	0·227	1·355	1·01	0·541	1·551
1/5	1·317	0·263	1·580	1·26	0·854	2·114
	1·802	0·383	2·185	1·24	0·777	2·017
1/10	0·766	0·216	0·982	1·12	0·426	1·546
	1·056	0·275	1·331	1·16	0·750	1·910
1/20	0·771	0·213	0·984	0·99	0·755	1·745
	0·947	0·308	1·255	0·81	0·740	1·550

In this series the strongest solution was too concentrated for the health of the plant, due doubtless to the higher temperatures and the considerable transpiration from the plant, which would still further concentrate the solution retained by the sand. The barley plants were also much affected by rust attacks.

Erratic as are the results shown by individual plants, there can be no doubt about the general superiority of the plants growing in solutions of higher concentration, as will be best seen by a comparison of the weights from the 1/5 and the 1/20 concentrations.

The whole series of experiments confirm the conclusion previously reached from the experiments described in Part I, that the concentration of the nutritive solution, within certain wide limits, irrespective of



the total amount of plant food available, is a factor in the rate of plant growth, which varies directly, though not proportionally, with the strength of the solution in the particular nutrient, or nutrients, limiting the growth[1].

III.—THE RATE OF DIFFUSION OF THE NUTRIENTS IN THE SOIL SOLUTION.

(A. D. H. and L. M. U.)

As was indicated in the introduction, it is conceivable that the nutrient in a soil solution may take so long to diffuse along the films coating the soil particles from the points where solution is effected to the root hairs that have exhausted the solution with which they are immediately in contact, that the plant may be continually running short of the food it could utilise. In order to test this hypothesis barley was grown in the same nutrient solutions (1) in a bottle as usual, (2) when diffused through a mass of pure sand in such a manner that the sand was nowhere saturated, but each particle was coated with a film of the solution. In the first case there will be no lag due to slowness of diffusion, because the liquid is being constantly mixed by convection currents, the daily aëration, etc., but in the second case the dissolved substances will have to travel to the roots in the surface film, sometimes for considerable distances, and the lag may become evident in a retardation of growth.

Cameron[2] has shown that for each soil there is a critical content of water that will induce a "crumb structure," in which state the soil contains no free water and is not sensibly wet, but can readily be crumbled without pasting. This condition can be found with fair accuracy by trial and corresponds to the optimum water content for growth. The sand used in the experiments was a uniform fine silver sand, the grains of which were above 0·2 mm. in diameter, and the critical water content corresponded to about one of water to five of sand. When wetted with this proportion of water the sand could be squeezed into a coherent mass but no water would exude, it also could be rubbed down into a fine crumb.

As the bottles held 600 c.c. of the culture solution, jars containing 3000 grm. of sand, through which 600 c.c. of the same solutions were diffused, were used in the comparisons; the same solutions varying in

[1] See also Pouget and Chouchak, *Compt. Rend.* 1912, Vol. 154, p. 1709.
[2] Bureau of Soils, U.S. Department of Agriculture, Bull. 50, 1908.

concentration from 1 to 1/20 were employed as in the preceding experiments, and barley was the trial plant. Every two or three days the jars were weighed and the original water content restored by the addition of pure water. Table XIII shows the results obtained (see Pl. VI, fig. 4).

TABLE XIII. *Comparative Growth of Barley in Sand and Water Cultures of Equal Concentration.*

Concentration of solution	Dry weight of plant in grammes	
	Water	Sand
1	1·655	7·050
	2·075	4·200
1/5	1·245	3·539
	1·492	3·031
1/10	1·030	3·171
	0·943	2·882
1/20	0·681	1·556
	0·537	1·437

It will be seen that so far from there being any retardation of growth in the sand due to slowness of diffusion of the nutrients in the water films, the sand cultures were markedly superior to the water cultures, though as before the rate of growth varies with the concentration of the nutrients in the solution. The experiment was repeated, and this time the nutrients in a concentrated solution were placed inside narrow cylinders of porous earthenware themselves filled with sand and packed in the sand in which the plant was growing. Thus the roots never came into contact with the nutrient solution until it had diffused through the porous cell and into the mass of sand beyond. The results are set out in Table XIV, from which it will be seen that the porous pot had introduced no new factor.

Again the growth varied with the concentration of the solution, and again the sand cultures were greatly superior to the corresponding water cultures.

There is thus no depression of growth due to slowness of diffusion of the nutrients in the water films on sand particles; it might be supposed, however, that the lag would become operative in the extended film that must exist on the far finer particles found in an ordinary soil.

Fig. 4.

Growth in sand containing solutions of varying concentration. Compare with Fig. 2

TABLE XIV. *Comparative Growth of Barley in Sand and Water Cultures of Equal Concentration. May 18—June 20, 1912.*

Concentration of solution	Dry weight of plant in grammes	
	Water	Sand
1	0·265 0·574	2·923 1·974
1/5	0·319 0·169	0·601 0·867
1/10	0·086 0·131	0·512 0·682
1/20	0·062 0·074	0·405 0·516

Accordingly a large quantity of sandy soil was graded into "coarse sand" as before, fine sand between 0·2 and 0·04 mm., and silt between 0·04 and 0·01 mm. Pure kaolin was taken to represent a clay material largely constituted of still finer particles. The critical water content was determined by trial as before, and the same solution was diffused through all the materials. Barley was grown five weeks with the following results:—

TABLE XV. *Growth of Barley in Nutrient Solution diffused over Solid Particles of various Grades.*

Nature of medium	Dry weight of plant in grammes		
	1	2	Mean
Water	1·350	1·190	1·270
Coarse sand	1·456	1·369	1·412
Fine sand	0·581	0·624	0·602
Silt	0·800	0·472	0·636
Kaolin	1·026	0·719	0·872

Through an accident the barley in the water cultures received twice the volume of nutrient solution that was diffused through the solid media; hence the comparison in this case was not exact. However, the sand culture preserved its superiority. In the media of finer grain there was evidently some factor at work depressing the growth, though

it would not appear to be the time required for diffusion, because it was least active in the kaolin, the finest medium of all.

The experiment was repeated with lupins with results set out in Table XVI.

TABLE XVI. *Growth of Lupins in Nutrient Solution diffused over Solid Particles of various Grades.*

Nature of medium	Dry weight of plant in grammes		
	1	2	Mean
Water	0·822	1·162	0·942
Coarse sand	2·486	2·462	2·474
Fine sand	0·896	1·367	1·131
Silt	1·416	1·371	1·393
Kaolin	1·742	1·925	1·833

On this occasion the growth in each of the solid media was superior to that in the same volume of solution in the free liquid state, so that the possibility of a retardation of growth due to slowness of diffusion may be dismissed. Some explanation is, however, required of the superiority of the cultures in sand over the water cultures, and, again, of the superiority of the cultures in coarse sand and kaolin over those in fine sand and silt. We were led to suspect that differences in the aëration of the roots might be the disturbing factor. The sand when properly wetted remains in a very open state, with large air spaces between the aggregates, and the roots could be observed traversing the whole medium freely; the kaolin preserved a very similar structure, whereas the fine sand and silt quickly settled down to a close mass. The appearance of the roots after they had been washed out of the sand, etc. (see Pl. VII, fig. 5), showed that they had been able to develop freely in the coarse sand and kaolin, but had been greatly restricted in the fine sand and silt.

Comparative water cultures were then arranged in which one series were not aërated at all, whereas in the other bottles a continuous current of air was bubbled through the solutions. The experiment was repeated with barley and lupins, and the results obtained are set out in Table XVII (see also Pl. VIII, fig. 6).

These results are convincing as to the enormous gain to the plant from continuous aëration of the root, and to this factor alone may be set

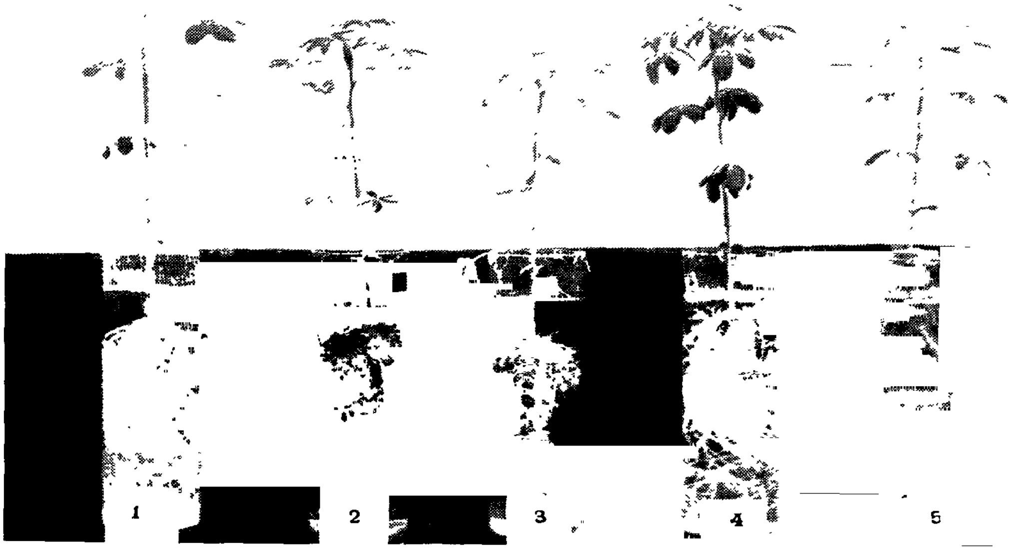


Fig. 5.

Appearance of Roots washed out of- 1. Silver sand. 2 Fine sand. 3. Silt. 4. Kaolin. 5. Water culture.

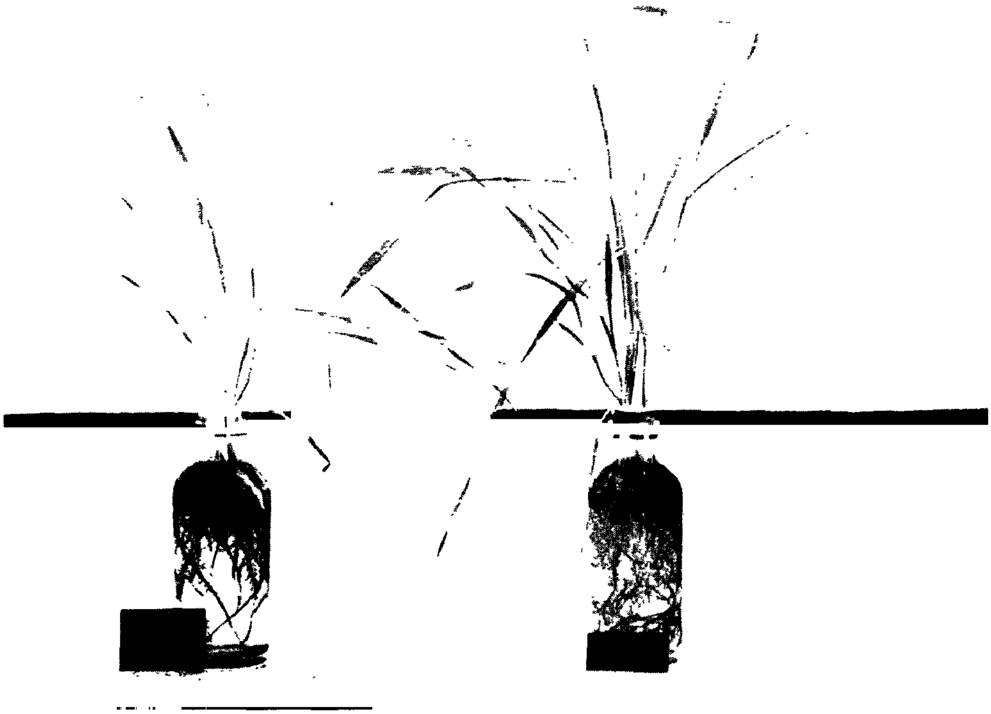


Fig. 6.

Growth of barley in solutions aerated once a day (left) and aerated continuously (right).

down the superiority of the cultures in solid media over the ordinary water cultures in which the aëration is not continuous.

TABLE XVII. *Growth of Barley and Lupins in Water Cultures variously aërated.*

Plant	Treatment of solution	Dry weight of plants in grammes				
		1	2	3	4	Mean
Barley	Not aërated	1·591	1·552	1·024	1·090	1·314
,,	Aërated continuously ..	2·528	1·646	2·159	2·156	2·122
Lupins	Not aërated	0·99	0·94	0·62	0·78	0·83
,,	Aërated once a day	0·73	0·67	0·68	0·79	0·72
,,	Aërated continuously ...	1·60	1·65	1·43	1·45	1·53

Finally, another set of experiments was tried with the coarse sand, silt, and kaolin, in which the water was added to the solid medium from below, so as to obviate as much as possible the settlement of the silt that had been so pronounced in the previous experiments. Settlement still occurred, but the growth made was more nearly equal (see Table XVIII), though the results are not conclusive as to whether aëration is the only factor concerned and whether the fine particles in the kaolin and silt are not holding back some of the nutrients from the plant by "adsorption."

TABLE XVIII. *Growth of Lupins in Solid Media, watered from below.*

Medium	Dry weight of plant
Sand	2·713
Sand	3·866
Silt	1·929
Kaolin	1·853
Kaolin	1·719

The difficulty of obtaining good crops on soils containing a large proportion of silt particles has repeatedly been observed; it may, in part, be set down to the ease with which the particles slip into a condition causing deficient aëration in the soil.

Summary and Conclusions.

Solutions were made by extracting the soils from certain of the Rothamsted plots on which wheat and barley had been grown for 60 years and upwards. Wheat and barley were grown in these solutions, which were renewed fortnightly. The comparative growth in the solutions was closely parallel to the growth of the crop on the plots in the field and corresponded to the composition of the solutions. The composition of the solutions as regards phosphoric acid and potash corresponded to the past manurial treatment of the soils and to the amount of phosphoric acid and potash they now show on analysis. Growth in the soil solutions agreed with the growth in artificial culture solutions containing equivalent amounts of phosphoric acid and potash. Growth in the soil solutions from imperfectly manured plots was brought up to the level of that in the solutions from completely manured plots on making up their deficiencies in phosphoric acid and potash by the addition of suitable salts. The phosphoric acid and potash content of the soil solutions was of the same order as the phosphoric acid and potash content of the natural drainage water from the same plots.

Wheat grew as well as barley in the solutions of the wheat soils, and *vice versâ*. In a similar set of solutions from the same soils the growth of buckwheat, white lupins and sunflowers corresponded with that of wheat and barley. Boiling effected no alteration in the nutritive value of the soil solutions

In nutritive solutions of various degrees of dilution the growth of plants varied directly, but not proportionally, with the concentration of the solution, though the total plant food present in the solution was in excess of the requirements of the plant. When the nutrient solution was diffused as a film over sand or soil particles, as in nature, there was no retardation of growth due to the slowness of the diffusion of the nutrients to the points in the liquid film which had been exhausted by contact with the roots. Growth in such nutrient solutions forming a film over sand particles was much superior to the growth in a water culture of equal concentration, but the growth in the water culture was similarly increased if a continuous current of air was kept passing through it.

From these data it is concluded:—

(1) The composition of the natural soil solution as regards phosphoric acid and potash is not constant, but varies significantly in accord with the composition of the soil and its past manurial history.

(2) Within wide limits the rate of growth of a plant varies with the concentration of the nutritive solution, irrespective of the total amount of plant food available.

(3) When other conditions, such as the supply of nitrogen, water, and air, are equal, the growth of the crop will be determined by the concentration of the soil solution in phosphoric acid and potash which, in its turn, is determined by the amount of these substances in the soil, their state of combination, and the fertiliser supplied.

(4) On normal cultivated soils the growth of crops like wheat and barley, even when repeated for 60 years in succession, does not leave behind in the soil specific toxic substances which have an injurious effect upon the growth of the same or other plants in that soil.

The net result of these investigations is to restore the earlier theory of the direct nutrition of the plant by fertilisers. The composition of the soil solution which determines the growth of the plant is dependent upon the amount and the mode of combination of the phosphoric acid and potash in the soil, both of which are affected by the fertiliser supply, though to what extent is not yet determinable.

THE RELATIVE EFFECT OF LIME AS OXIDE AND CARBONATE ON CERTAIN SOILS.

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In an earlier communication by one of us (1) an account was given of some preliminary experiments on the action of caustic lime on the soil, with particular reference to the phenomena of partial sterilisation.

From the practical standpoint the question is of considerable importance, inasmuch as an effective sterilising agent for use in intensive field cultivation has yet to be found. Caustic lime, however, has other effects: it is used for promoting more rapid decay, thus ensuring a quicker circulation of foodstuffs in the soil, and employed for destroying soil pests and for "sweetening" soil. Little, however, is known of the mechanisms of these changes, and the present extended investigation was undertaken to study the subject from a broader point of view.

Schulze in 1846 (2) found that an application of caustic lime to a humus soil accelerated the consumption of oxygen and the production of carbon dioxide in a marked degree. Similar results were recorded by Peterson (3) and at a later date and with reference to carbonate, by Ebermayer (4) and also Hilgard (5), while the effect of caustic lime in destroying organic matter of the soil has been demonstrated by Wheeler, Sargent and Hartwell (6) amongst others. Boussingault observed that the conversion of unavailable nitrogen to ammonia was greatly facilitated by applications of lime, while the production of nitrates was inhibited. The type of action on micro-organic growth in

the soil depends largely on the compound added. Calcium oxide not only neutralises soil acidity but also appears to break down some organic compounds and increases the amount of compounds easily resolved into ammonia or capable of serving as food for bacteria. Calcium carbonate, on the other hand, appears only to neutralise acid soil compounds and not to decompose the complex organic residues. This difference accounts for many of the experimental results obtained.

Chester (7) records increases in the number of organisms in certain limed soils, and concludes that this effect was not due to any direct action of the lime but to the more favourable reaction induced in the soil. In field experiments by Fabricius and von Feilitzen (8) an application of lime greatly increased the number of bacteria in moor soils, those in their natural condition containing only relatively few organisms.

Engberding (9) records a temporary rise in the numbers of bacteria following treatment of a soil with 0·1 per cent. calcium oxide. This is in accordance with the results already recorded by us.

Fischer (10) added lime to soils and observed first an appreciable initial depression in the numbers of bacteria and then a rapid rise to very high numbers. This was regarded by the author as a pure stimulation effect, although we consider it was due to the production of available food stuffs by the lime.

Wolf (11) observed the favourable effect of lime on the production of nitrates, and this was confirmed at a later date by Peterson (3) and numerous others. The increased availability of certain fertilisers as the result of liming has been shown to be effected with raw bone by Jenkins and Britton (12) and with dried blood, cotton seed meal and other organic residues by Withers and Fraps (13).

The effect of certain additions of lime on nitrification as also on nitrogen fixation, etc., has been further demonstrated. Wohltmann, Fischer and Schneider (14), examining field soils which had been limed, found that the amount of nitrate and the available potash and phosphate were increased in consequence.

The importance of the presence of lime in the soil for nitrogen fixation has been shown by Ashby, working in this laboratory, who found that a deficiency in this soil constituent led to a decrease in nitrogen-fixing powers, while the susceptibility of the free-living nitrogen-fixing organism Azotobacter has been suggested as a test for the presence of alkaline carbonates or calcium carbonate in the soil (15). The function of lime in increasing the biochemical efficiency

of bacteria has been demonstrated in numerous instances by means of the Remy-Löhnis method, and work on these lines has indicated an improvement in soil conditions for such processes as peptone decomposition, nitrification and nitrogen fixation (16).

In the earlier paper, to which reference has already been made, it was shown that lime in the caustic state exerted in common with other mild antiseptics a specific action by disturbing the biological equilibrium in the soil. As a result of this, certain classes of protozoa, in common with the nitrifying bacteria, were destroyed by heavy applications of lime; after an initial depression, the total numbers of bacteria growing on gelatine plates were subsequently largely increased, and led to corresponding increments of ammonia, and, in pot experiments, of plant growth. In continuation of this work we have attempted to determine the amount of lime required to induce partial sterilisation, and the relative values of calcium oxide and carbonate in other and acid soils, and further, to determine the general character of the changes set up in each case.

EXPERIMENTAL.

The investigations were carried out with five soils of widely different types. Their general characters are detailed below and, in some cases, information as to their behaviour towards lime under field conditions is available.

1. *Rothamsted Soil.* This is a poor unmanured soil from the paths on Hoos Field, it is a stiff clay loam overlying chalk and containing 2·22 per cent. carbonate.

2. *Chelsea Soil.* From the Physic Gardens, Chelsea, S.W. This soil was originally Thames Sand, but by heavy applications of stable manure for many generations and by deposition of soot it has become highly organic in character and black in colour.

3. *Craibstone Soil.* From the Experimental Farm of the North of Scotland College of Agriculture. It is a light sandy organic soil deficient in carbonate but regarded as being comparatively fertile.

4. *Millbrook Soil.* From a portion of the Woburn Experimental Fruit Farm. It is a very light sandy soil, low in organic matter, and was selected on this account to indicate how far injurious results might attend heavy dressings of caustic lime.

5. *Woburn Soil.* From the Woburn Experimental Station of the Royal Agricultural Society. The soil is an open sandy loam, and has

been rendered distinctly acid by annual applications of sulphate of ammonia for the last 37 years. This acidity is so pronounced in the field as to check the growth of wheat and barley but not of oats.

TABLE I. *Showing the percentages of organic matter, nitrogen, etc., in the various soils.*

	Loss on ignition	Nitrogen	Carbonate as $CaCO_3$	CaO	MgO	Clay
Rothamsted Soil	7·72	0·133	2·66	1·94	0·21	1·39
Millbrook ,,	4·46	0·085	0·03	0·26	0·15	0·59
Chelsea ,,	14·32	0·455	0·89	1·42	0·17	0·63
Craibstone ,,	14·55	0·316	none	0·41	0·22	0·42
Woburn ,,	4·58	0·117	none	0·56	0·15	0·63

In order that the changes following treatment might be determined as closely as possible, monthly examinations of the various soils were carried out. The numbers of bacteria were ascertained by the gelatine plate method, and the presence of protozoa tested by inoculation of a sample of each soil into hay infusion. Free ammonia in the soils was estimated by the method described by Russell[1] and the nitrates determined after reduction by the zinc-copper couple.

Before treatment all the soils were passed through a 3 mm. sieve and filled into bottles in lots of 900 grms. A set of bottles from each soil was set aside as control, and the others received calcium oxide in the proportion of 0·1, 0·2, 0·3, 0·4, 0·5 and 1·0 per cent., while calcium carbonate was added to another lot to the extent of 1 per cent., with the exception of the Craibstone soil, where applications of 0·3, 0·6, 0·9 per cent. of carbonate were made. The water content was made up, by means of sterilised water, to 18 per cent. A set of bottles was taken immediately after the end of this ten day treatment, and the others were stored provided with cotton wool plugs at room temperature.

Rothamsted Soil.

As shown above, this soil already contained sufficient calcium carbonate for normal growth, and further additions fail to induce any change in bacterial growth, production of plant food, or weight of crop. All the applications of quicklime, with the exception of 0·1 per cent., caused an initial depression in the numbers of bacteria. In the case of the lightest application the bacteria rose within the first ten days from

[1] Russell, This *Journal*, 1910, **3**, 233.

23 to 37 millions, and then decreased gradually to the level in the untreated soil. Thirty days afterwards the bacteria in the soil receiving 0·2 per cent. calcium oxide had risen to 166 millions and then began to decrease. Somewhat similar changes occurred in the soils receiving heavier dressings, and in general the heavier the application the greater is the initial depression in bacterial numbers, and the longer the period before the numbers for each particular dressing attain a maximum. The soil receiving 1·0 per cent. of caustic lime showed no material increase in numbers even after 120 days. Further, the addition of a fairly large quantity of carbonate failed to affect the bacterial numbers. The high numbers obtained with some of these soils, for instance after 90 days with 0·5 per cent. of calcium oxide, were chiefly a yellow micro-coccus, which disappeared within a short time and presumably flourished on some of the decomposition products resulting from the action of the lime on the soil.

The direct chemical action of the lime results in an immediate increase in the amount of free ammonia, and especially in the case of the heaviest dressing, where during the period of bacterial inaction the amount of ammonia increased from 3 to 24 parts per million, whilst when the bacteria were active it rose from 24 to 92 parts per million. The very high bacterial numbers observed after 90 days are not, however, associated with any corresponding increase of ammonia; indeed, where 0·5 per cent. of lime was added, no increase was obtained between the 40th and the 120th day. Initial inhibition of nitrification is apparent with dressings above 0·1 per cent. calcium oxide, and permanent inhibition with 0·4 per cent. Tests for protozoa showed the presence of some of the larger forms in the control, and the two soils receiving the lower applications of lime. After 40 days they appeared in soil receiving 0·3 per cent. and were absent from the soils receiving heavier doses, while present in that receiving carbonate.

These two facts indicate that the partial sterilisation effect is produced by dressings of 0·3 per cent. or more calcium oxide. But where a solid and non-volatile antiseptic is employed it is difficult to discriminate sharply between the partial sterilisation and other effects.

Millbrook Soil.

This light sandy soil exhibits a greater sensitiveness towards relatively small applications of caustic lime than the Rothamsted soil. Not only is the initial depression in bacterial numbers greater, but the effect appears to persist longer. With the lighter doses such as 0·1 and

TABLE II. *Showing the effect of calcium oxide and of carbonate on numbers of bacteria and the production of ammonia and nitrate in Rothamsted soil.*

(*a*) Bacteria

Treatment	Millions per gram of dry soil after							
	10 days	40 days	60 days	90 days	120 days	160 days	200 days	260 days
Control soil	23	20	15	17	11	14	7	11
Soil + 0·1 % CaO	37	32	21	44	18	14	7	13
Soil + 0·2 ,,	12	116	49	101	32	73	24	23
Soil + 0·3 ,,	6	62	61	104	43	30	42	34
Soil + 0·4 ,,	5	32	36	247	100	46	45	75
Soil + 0·5 ,,	3	16	41	474	22	24	41	41
Soil + 1·0 ,,	2	1	1	3	4	24	72	27
Soil + 1·0 % $CaCO_3$	22	21	18	12	14	10	7	11

(*b*) Ammonia and Nitrate

Treatment	Expressed as parts of nitrogen per million of dry soil														Gain in nitrogen as ammonia and nitrate after 260 days
	Nitrogen present as ammonia after							Nitrogen present as nitrate after							
	10 days	40 days	60 days	90 days	120 days	200 days	260 days	10 days	40 days	60 days	90 days	120 days	200 days	260 days	
Control soil	3	2	1	1	3	3	1	11	12	12	13	14	18	24	11
Soil + 0·1 % CaO	3	2	1	1	2	2	1	11	18	22	24	26	32	33	20
Soil + 0·2 ,,	3	11	3	5	3	3	1	10	12	20	29	43	49	56	43
Soil + 0·3 ,,	8	15	12	14	12	12	4	9	12	13	11	17	45	48	38
Soil + 0·4 ,,	10	16	12	24	30	34	37	11	12	10	9	12	24	19	42
Soil + 0·5 ,,	12	26	32	27	25	54	44	11	12	12	10	13	12	17	47
Soil + 1·0 ,,	13	20	15	17	25	67	93	17 (?)	14	12	14	13	13	13	92
Soil + 1·0 % $CaCO_3$	2	2	2	1	2	2	2	13	16	12	13	16	18	17	5

0·2 per cent. calcium oxide, however, there is a quick recovery within the first 10 days, and the bacterial content rises to about 77 million. This we suppose to be due to a temporary feeding effect, as is also the high content observed at the end of 30 days with the 0·3 per cent. application. The application of carbonate to this soil, comparatively poor in lime, is not followed by any appreciable increase in the numbers of bacteria for the first few months, although the production of nitrates appears to be increased slightly.

The initial amount of ammonia produced by the action of 0·1 per cent. calcium oxide was almost completely nitrified within the first month, whilst the effect of 0·2 per cent. persisted for a month longer. A greater retardation by 0·3 per cent. persisted up to four months, and at the end of this time nitrification set in. All larger dressings completely inhibited the process; in fact a slight loss of nitrate seems to have occurred. As already pointed out, the effect of the carbonate is slight but definite. Examinations for protozoa after 30 days showed the absence of ciliates from all the soils receiving more than 0·2 per cent. calcium oxide, and of all forms when 0·5 and 1 per cent. were given.

Chelsea Soil.

As compared with the behaviour of the two foregoing poor soils, that of rich soils such as Chelsea, containing an abundance of carbonate, or Craibstone, deficient in carbonate, is very striking. Table IV shows that even the heaviest application of calcium oxide failed to reduce the numbers of bacteria to any great extent in the Chelsea soil. The numbers show considerable variation, but in general they increase up to about 70 days, and then steadily fall off. Finally, after 10 months, they are roughly proportional to the amount of caustic lime applied. Carbonate had little or no effect. No permanent depression of nitrification occurred, the heavy application of 1·0 per cent. calcium oxide only checked it for the period of 130 days. However, examinations for protozoa immediately after treatment failed to reveal the presence of ciliated protozoa in the soils receiving 0·5 and 1·0 per cent. calcium oxide, but after 130 days flagellates only were found in the latter soil, indicating that about this point some measure of partial sterilisation had occurred. In the case of this soil, as in the two preceding ones, we find the usual equal sensitiveness of nitrifying bacteria and of the higher protozoa towards treatment.

TABLE III. *Showing the effect of calcium oxide and of carbonate on numbers of bacteria and the production of ammonia and nitrate in Millbrook soil.*

(*a*) Bacteria

Treatment	Millions per gram of dry soil after				
	10 days	30 days	70 days	100 days	130 days
Control soil	14	11	10	9	9
Soil + 0·1 °/ₒ CaO	57	33	30	22	19
Soil + 0 2 ,,	77	84	114	35	23
Soil + 0·3 ,,	3	481	107	58	78
Soil + 0·4 ,,	1	66	98	29	98
Soil + 0·5 ,,	1	·4	—	30	—
Soil + 1·0 ,,	·3	·1	·1	21	5
Soil + 1·0 °/ₒ $CaCO_3$	14	14	10	10	12

(*b*) Ammonia and Nitrate

Treatment	Expressed as parts of nitrogen per million of dry soil										Gain in nitrogen as ammonia and nitrate after 130 days
	Nitrogen present as ammonia after					Nitrogen present as nitrate after					
	10 days	30 days	70 days	100 days	130 days	10 days	30 days	70 days	100 days	130 days	
Control soil	2	1	4	3	2	9	10	12	—	13*	4
Soil + 0·1 °/ₒ CaO	7	1	4	3	1	11	19	19	19	28	18
Soil + 0·2 ,,	12	13	5	4	2	9	10	31	38	38	29
Soil + 0·3 ,,	10	16	30	28	7	10	10	8	19	36	32
Soil + 0·4 ,,	10	14	36	48	45	12	10	8	—	6	40
Soil + 0·5 ,,	11	12	35	53	53	9	10	10	8	6	48
Soil + 1·0 ,,	17	13	19	28	52	10	11	13	15	8	49
Soil + 1·0 °/ₒ $CaCO_3$	2	1	4	3	2	9	10	17	17	20	11

Craibstone Soil.

In some respects this soil resembles that from Chelsea; both are light, rich in organic matter, and poor in clay, but while the Chelsea soil contains upwards of 1·0 per cent. calcium carbonate the Craibstone soil contains very little.

The remarkable difference is that the smaller applications of lime do not appreciably affect the bacterial numbers, and 1·0 per cent. caused only a small initial depression, which persists for a short period only. In no instance are such high counts recorded as with other soils, and any depression of the higher protozoa appears to pass off within the

first few weeks after treatment. The addition of various amounts of calcium carbonate[1] failed to induce increased growth of bacteria, presumably because there was no disturbance of the equilibrium between these and other organisms. The total flora, on the other hand, appeared to function more efficiently, and there was a greatly increased nitrate production.

The amount of ammonia produced immediately after treatment is not very great, and it usually disappears within 40 days. Only in the case of the 1·0 per cent. oxide application is there any accumulation of ammonia, and this begins to disappear after about 80 days. In spite of the low carbonate content of the untreated soil, nitrification is active from the very first, but is decidedly accelerated by all the lime applications. The relative return of nitrates for equivalent amounts of calcium or oxide of carbonate would appear to be greater with the former within the first 170 days; between this and 380 days the difference becomes eliminated.

Woburn Soil.

The Woburn soil stands out in striking contrast to these. As a result of its previous annual applications of ammonium sulphate it has been depleted of all carbonate, and possesses a well-marked acid reaction.

Table VI shows that no depression in bacterial numbers is produced at the end of 10 days excepting in the soil receiving 1·0 per cent. calcium oxide. The results suggest an almost immediate absorption of all the lower doses of caustic lime, and simultaneous access to nutrients hitherto held out of action by adverse conditions. The application of 0·1 per cent. calcium oxide has sufficed to suspend temporarily the action of these conditions, but at the end of 200 days there is a return in numbers to the level of those in the control soil. The larger dressings apparently give rise to a feeding effect, and very high numbers (906 millions) are obtained after 90 days with soil 0·4 per cent. CaO. Furthermore, the occurrence of greater numbers of bacteria in this soil than in that receiving 0·5 per cent. is due no doubt to the extinction of less resistant but more actively growing bacteria in the latter[2]. With

[1] With this soil the scheme of experiment was varied by applying three different weights of calcium carbonate for purposes of comparison with the caustic lime.

[2] This would appear to constitute a parallel with effects observed in other work. Treatment of a soil with toluene serves to kill off certain species of bacteria, which when reintroduced to a treated soil give much higher numbers than are observed in toluened soils not reinfected.

TABLE IV. *Showing the effect of calcium oxide and of carbonate on numbers of bacteria and the production of ammonia and nitrate in Chelsea soil.*

(*a*) Bacteria

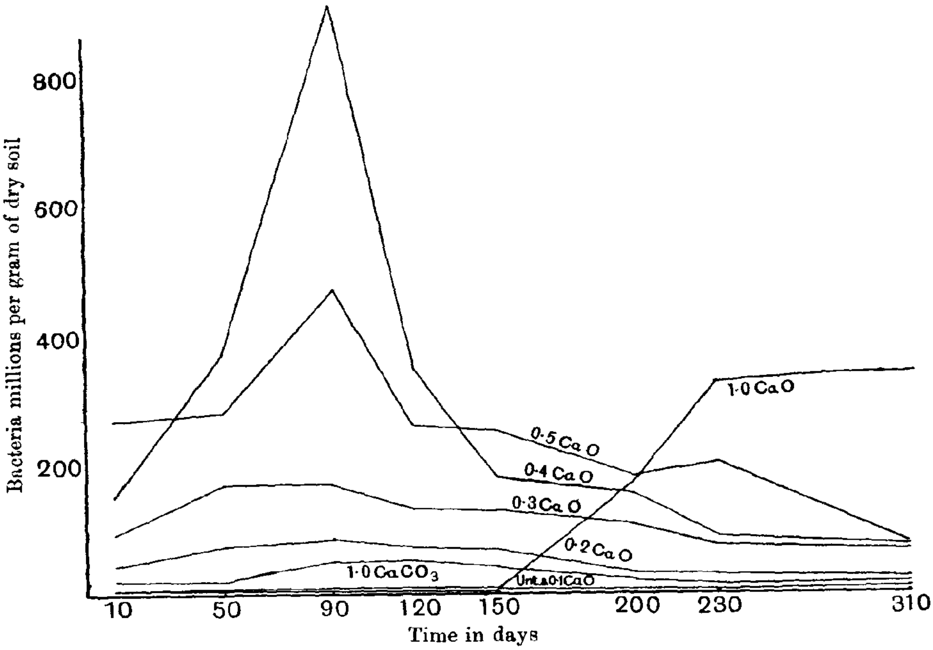
Treatment	Millions per gram of dry soil after							
	10 days	40 days	70 days	100 days	130 days	160 days	200 days	280 days
Control soil	11	30	31	17	20	13	16	14
Soil + 0·1 °/$_o$ CaO	9	19	41	19	16	12	15	11
Soil + 0·2 ,,	10	27	40	22	28	21	18	16
Soil + 0·3 ,,	9	44	80	25	62	25	22	20
Soil + 0·4 ,,	7	42	87	60	97	27	32	31
Soil + 0·5 ,,	7	43	325	45	66	38	39	50
Soil + 1·0 ,,	5	26	225	18	20	49	139	75
Soil + 1·0 °/$_o$ $CaCO_3$	9	30	16	16	36	13	13	13

(*b*) Ammonia and Nitrate

Treatment	Expressed as parts of nitrogen per million of dry soil												Gain in nitrogen as ammonia and nitrate after 280 days
	Nitrogen present as ammonia after						Nitrogen present as nitrate after						
	10 days	40 days	70 days	100 days	130 days	280 days	10 days	40 days	70 days	100 days	130 days	280 days	
Control soil	9	6	8	6	7	7	64	69	85	90	96	115	49
Soil + 0·1 °/$_o$ CaO	13	4	10	7	6	6	67	65	99	99	102	128	61
Soil + 0·2 ,,	15	7	8	6	3	7	71	70	95	108	109	132	66
Soil + 0·3 ,,	15	5	6	6	3	5	56	57	102	115	116	148	80
Soil + 0·4 ,,	15	5	9	6	5	5	47	69	102	123	122	152	84
Soil + 0·5 ,,	15	29	11	6	8	6	64	65	110	100	122	166	99
Soil + 1·0 ,,	27	36	44	54	75	12	57	55	71	74	71	177	116
Soil + 1·0 °/$_o$ $CaCO_3$	7	2	9	7	5	8	66	69	80	74	101	109	44

the heaviest dressing of caustic lime the depression in numbers persists for upwards of 150 days, but then a progressive increase occurs at least up to the end of 300 days.

The data obtained from the determinations of ammonia are interesting. The initial amount of ammonia is high on account of previous treatment and the acidity of the soil, but in spite of this a slow change to nitrate occurs as well as additional breakdown processes, since the ammonia is reduced by 9 parts per million and the total production



Curve 1. Bacterial numbers in Woburn soil.

of ammonia and nitrate is increased by 40 parts. Small quantities of caustic lime or of carbonate bring about a rapid decrease in the amount of free ammonia, but larger quantities do not. Only the heaviest dressing, however, retarded the change for any considerable time. The total gain in nitrogen, as ammonia and nitrate, is much greater per increment of 0·1 per cent. calcium oxide than in any of the other soils. Ciliates, amoebae and flagellates were found in all the soils excepting that receiving 1·0 per cent. calcium oxide, in which amoebae only were found after 10 days, but at all subsequent examinations only flagellates appeared. Here again parallelism between disappearance of protozoa and of nitrifying organisms occurs.

TABLE V. *Showing the effect of calcium oxide and of carbonate on numbers of bacteria and the production of ammonia and nitrate in Craibstone soil.*

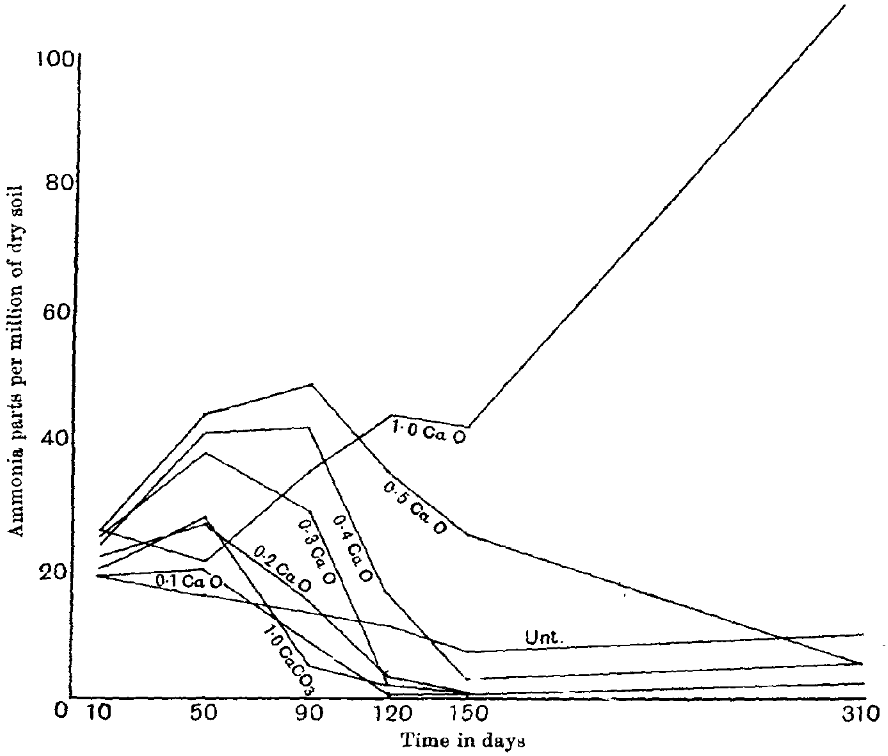
(*a*) Bacteria

Treatment	Millions per gram of dry soil after						
	10 days	40 days	80 days	110 days	140 days	170 days	210 days
Control soil	14	10	12	9	12	12	13
Soil + 0·1 % CaO	16	32	15	8	13	7	10
Soil + 0·2 ,,	13	14	15	8	14	6	8
Soil + 0·3 ,,	10	44	18	14	14	7	10
Soil + 0·4 ,,	20	53	29	17	22	16	13
Soil + 0·5 ,,	11	40	51	25	27	24	20
Soil + 1·0 ,,	4	42	41	54	64	41	60
Soil + 0·3 % $CaCO_3$	10	14	11	8	16	5	7
Soil + 0·6 ,,	11	14	10	8	9	8	12
Soil + 0·9 ,,	10	14	13	8	11	10	12

(*b*) Ammonia and Nitrate

Treatment	Expressed as parts of nitrogen per million of dry soil													Gain in nitrogen as ammonia and nitrate after 170 days
	Nitrogen present as ammonia after						Nitrogen present as nitrate after							
	10 days	40 days	80 days	110 days	140 days	170 days	10 days	40 days	80 days	110 days	140 days	170 days	3[illegible]0 days	
Control soil	2	4	3	5	4	5	21	26	28	34	43	44	67·5	26
Soil + 0·1 % CaO	1	3	3	5	2	5	24	31	31	37	40	49	90·6	31
Soil + 0·2 ,,	1	4	3	5	3	5	25	33	34	42	47	50	95·5	32
Soil + 0·3 ,,	6	4	2	4	3	4	27	37	38	45	55	64	100·1	45
Soil + 0·4 ,,	8	4	3	4	3	4	21	43	47	56	53	77	115·8	58
Soil + 0·5 ,,	9	9	3	5	4	4	23	39	50	67	77	88	136·8	69
Soil + 1·0 ,,	13	32	55	35	11	4	24	25	27	68	101	151	210·0	132
Soil + 0·3 % $CaCO_3$	2	1	4	5	2	4	28	30	34	46	44	53	—	34
Soil + 0·6 ,,	1	2	4	4	4	4	32	34	35	45	56	59	119·0	40
Soil + 0·9 ,,	2	2	4	4	2	4	26	38	38	45	55	66	148·1	47

Reviewing the foregoing results, it is obvious that the addition of caustic lime to these soils exercised a marked effect on the growth of putrefactive and nitrifying organisms and on soil protozoa. This action leads to a simplification of the soil flora, and in some cases is sufficient to alter the character of the nitrogen compounds available as food for plants, ammonia production being increased and that of nitrates depressed in the primary stages of growth. The increases in total



Curve 2. Ammonia production in Woburn soil.

ammonia and nitrates for each increment of lime show a certain proportionality with the amount applied, but this varies with the character of the reserves of soil nitrogen and the comparative poverty of the soil in carbonate. The Woburn soil is typical of the first case, where considerable reserves are liberated on the application of small doses of lime; with the Craibstone soil the lime is directly absorbed by the soil, and the comparative returns are low. It may be stated, however, as a general average, that the relative return of nitrogen in a form

TABLE VI. *Showing the effect of calcium oxide and of carbonate on numbers of bacteria and the production of ammonia and nitrate in Woburn soil.*

(*a*) Bacteria

Treatment	Millions per gram of dry soil after							
	10 days	50 days	90 days	120 days	150 days	200 days	232 days	310 days
Control soil	5	5	4	4	4	4	5	5
Soil + 0·1 % CaO	6	11	11	9	10	4	5	3
Soil + 0·2 ,,	23	78	82	68	73	29	19	13
Soil + 0·3 ,,	97	170	170	132	133	106	77	63
Soil + 0·4 ,,	151	387	906	345	178	154	87	71
Soil + 0·5 ,,	270	281	470	260	247	180	200	77
Soil + 1·0 ,,	2	·2	6	7	11	176	329	337
Soil + 1·0 % $CaCO_3$	9	18	52	55	40	33	27	11

(*b*) Ammonia and Nitrate

Treatment	Expressed as parts of nitrogen per million of dry soil												Gain in nitrogen as ammonia and nitrate after 310 days
	Nitrogen present as ammonia after						Nitrogen present as nitrate after						
	10 days	50 days	90 days	120 days	150 days	310 days	10 days	50 days	90 days	120 days	150 days	310 days	
Control Soil	19	16	15	11	7	10	5	15	19	23	29	54	40
Soil + 0·1 % CaO	19	20	8	1	1	3	11	27	38	48	52	79	58
Soil + 0·2 ,,	22	27	15	3	1	3	10	23	38	61	67	84	63
Soil + 0·3 ,,	25	38	29	2	1	4	12	21	29	66	70	102	82
Soil + 0·4 ,,	24	41	42	16	3	5	11	14	21	49	74	111	92
Soil + 0·5 ,,	26	44	49	35	25	5	13	12	17	29	48	116	97
Soil + 1·0 ,,	26	21	35	44	42	112	12	19	13	14	16	16	104
Soil + 1·0 % $CaCO_3$	20	28	5	2	1	4	9	23	51	60	71	104	84

available for plants, within the first 8—9 months, is approximately 1 per cent. of the caustic lime applied; details are given in the following table, where the difference in ammonia and nitrate content of the untreated and each of the treated soils is given in terms of increments of 0·1 per cent. calcium oxide. The data for the different soils are not for directly comparable periods, but are the final figures obtained in each case, as given in the detailed ammonia and nitrate tables.

TABLE VII. *Increments of ammonia and nitrate with various applications of lime.*

Treatment	Rothamsted			Millbrook			Chelsea			Craibstone			Woburn		
	Initial	Final	Increment	Initial	Final	Increment	Initial	Final	Increment	Initial	Final	Increment	Initial	Final	Increment
Untreated	14	25	—	11	15	—	73	122	—	23	72	—	24	64	—
0·1 °/ₒ CaO	14	34	9	18	29	14	80	134	12	25	95	23	30	82	18
0·2 ,,	13	57	16	21	40	12	86	139	9	26	100	14	32	87	12
0·3 ,,	17	52	9	20	43	9	71	153	10	33	104	11	37	106	14
0·4 ,,	21	56	8	22	51	9	62	157	9	29	119	12	35	116	13
0·5 ,,	23	61	7	20	59	9	89	172	10	32	140	14	39	121	11
1·0 ,,	30	106	8	27	60	4	84	189	7	37	214	14	38	128	6
1·0 °/ₒ CaO_3	15	19	—	11	22	1	73	117	—	28	152	14	29	108	8

As to the fate of the lime added, fresh evidence will be adduced in a later paper to show that even in soils initially rich in carbonate its reappearance as carbonate is a matter of extreme slowness. Hence the determination of carbonate in a soil recently limed would be quite misleading as to conditions obtaining therein.

POT EXPERIMENTS.

These have been carried out with all except the Woburn soil. The soils were passed through a 3 mm. sieve previous to being filled into glazed earthenware pots (the bottom tubulure of which was corked up in order to prevent loss by drainage), and the amount taken was such as to bring the surface of the soil to within an inch or so from the upper rim of the pot. The weight required consequently varied somewhat with the different soils, 9 kg. of Craibstone soil, 8 kg. of Chelsea

soil, 10 kg. of Millbrook soil and 10 kg. of Rothamsted soil[1] being taken. Lime as oxide and carbonate was applied in the same ratio as in the laboratory experiments. The surface soil was then wetted with 100 c.c. of water in order to allow of efficient action of the caustic lime before it became carbonated. Ten barley seeds were sown in each pot, and after about a month the plants were thinned out to five per pot. Equal numbers of the seedlings were weighed to ascertain the initial effect of the lime on plant growth.

Rothamsted Soil.

The soil was treated on Jan. 15th, 1913, and the barley sown on April 11th; the seedlings were thinned out on May 15th, and the crop, cut on July 15th, was slightly, but as far as could be judged, evenly damaged by birds. Germination in all the soils was regular, but at the time of thinning, those plants in soils with 0·1 and 0·2 per cent. calcium oxide were slightly better than those in the control soil. The higher, especially the highest dressings of oxide, appeared to be injuring growth, causing the tips of the leaves to wither.

Table VIII shows the yields of dry produce, etc. A second crop of mustard was then grown in all the pots, and harvested on Oct. 2nd. The yields of dry matter were greatly increased by the applications of small dressings of caustic lime, and reached a maximum with 0·3 per cent. The next higher dressing caused a slight decrease, while the crop growing in soil with 1·0 per cent. oxide was only 14·0 per cent. of that in the control soil. The addition of carbonate was without effect. The second crop, mustard, showed on the whole progressive increases up to the heaviest lime dressing, and carbonate also appeared to cause a slight increase. The production of dry matter in the two crops also increases to the 0·3 per cent. application, and then declines. The percentages of nitrogen in the dry matter increase with the lime dressings, indicating non-nitric nutrition of the plants. The total nitrogen in both crops shows a steady increase, and not a decrease, with higher lime dressings.

[1] For this soil results showing the effects of lime on plant growth in pot culture and in the field are available elsewhere. Voelcker, J. A., "Annual Reports of the Consulting Chemist." *Journ. Roy. Agric. Soc.* 1898—1913.

On account of its stiff clayey nature the Rothamsted soil was mixed with one-third of its weight of clean sand. The dressings of lime were however calculated on the basis of soil only 1, 3, 7, 5 Ko.

Millbrook Soil.

The pots in this set were put up and the soil treated on March 17th, the barley being sown on May 2nd and afterwards thinned out in the usual way. Germination was good in all cases, with the exception of the heaviest limed soil (Plate IX, figs. 1 and 2). The results are very similar to those described above, except for the greater sensitiveness towards higher doses of caustic lime. A maximum is reached with 0·3 per cent. oxide, while 1·0 per cent. almost checks growth completely. The recovery in the second crop is almost complete, soils with 1·0 per cent. oxide showing a decrease as compared with those receiving the next lower dressing. In some cases the physical condition of the Millbrook soil was altered to such an extent by liming that the seedlings of the first crop were suffering from lack of water, while the soil 10 to 12 inches below was waterlogged.

Craibstone Soil.

The soil was treated and filled into pots on Jan. 16th and barley sown on April 11th. No differences in germination between the various sets could be detected, and the weights of the seedlings removed at the time of thinning, a month later, show decided increases in weight in all cases as compared with the control. Growth was vigorous throughout, and the crop was cut on July 15th. Progressively increasing yields of dry matter were obtained in all cases in the first and second crop, and the beneficial effect of carbonate is well marked. The magnitude of the crops and the nitrogen content, obtained with dressings of 0·1 to 0·3 per cent. calcium oxide, are very similar to those obtained with carbonate. Comparable results are also found in the case of the second crop.

Chelsea Soil.

The soil was treated on March 3rd and sown with barley on May 2nd. Germination was quite even, and the weights of seedlings at the time of thinning, on May 27th, showed slight increases, with the exception of those from soils receiving 1·0 per cent. oxide and 1·0 per cent. carbonate. The crop results are similar to those obtained previously with garden soil[1] (Plate X, figs. 1 and 2). 0·2 per cent. calcium oxide slightly increases the yield, but higher doses tend to decrease it. This is not due to the persistence of caustic lime; indeed lime disappears more rapidly from this soil than from the lighter ones, as is also

[1] Hutchinson, *loc. cit.* p. 327.

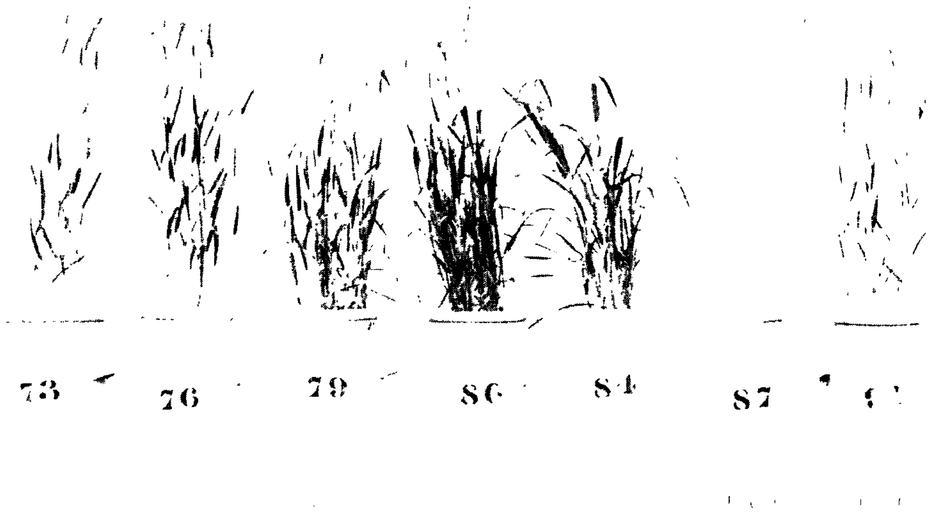


Fig. 1. First crop, Barley.

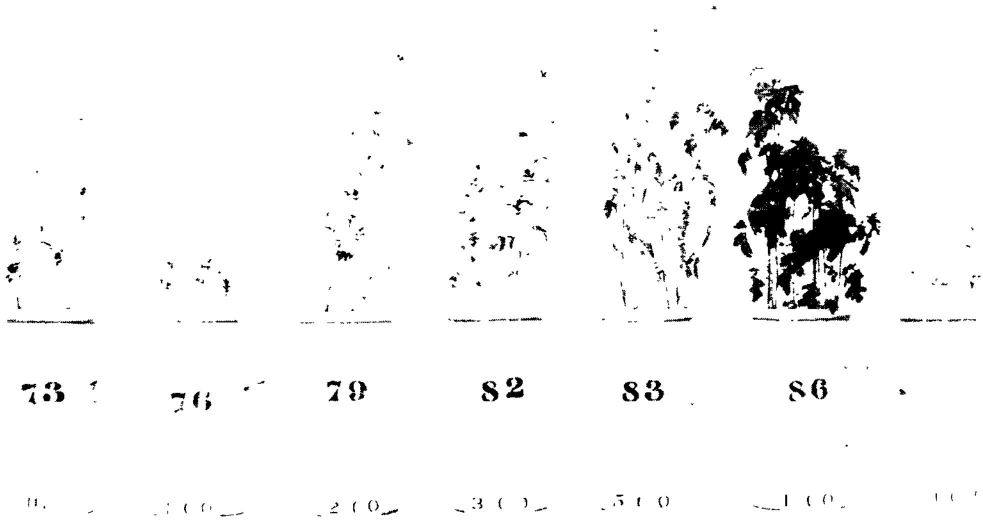


Fig. 2. Second crop, Mustard.

Experiments on the action of Caustic Lime and Carbonate on Millbrook Soil. Treatment (from left to right). Untreated soil, and soil with the addition of 0·1, 0·2, 0·3, 0·5 and 1·0 per cent. caustic lime and 1·0 per cent. chalk respectively.

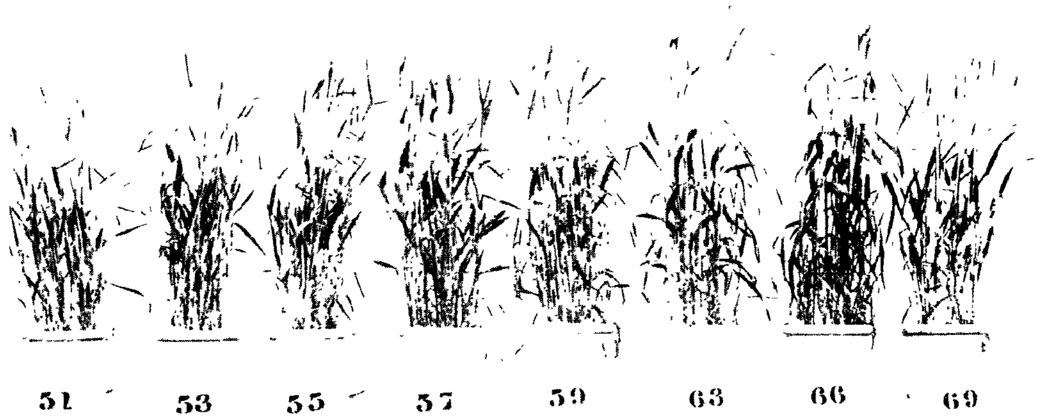


Fig. 1. First crop, Barley.

Fig. 2. Second crop, Mustard.

Experiments on the action of Caustic Lime and Carbonate on Chelsea Soil. Treatment (from left to right). Untreated soil, and soil with the addition of 0·1, 0·2, 0·3, 0·4, 0·5 and 1·0 per cent. caustic lime and 1·0 per cent. chalk respectively.

TABLE VIII. *Showing the results of pot experiments with various untreated and limed soils.*

Soil and Treatment	1st crop Barley: Dry matter grams	1st crop Barley: Relative weights	1st crop Barley: Nitrogen in crop per cent.	1st crop Barley: Total nitrogen in crop grams	2nd crop Mustard: Dry matter grams	2nd crop Mustard: Relative weights	2nd crop Mustard: Nitrogen in crop per cent.	2nd crop Mustard: Total nitrogen in crop grams	Total dry matter in both crops, grams	Total nitrogen in both crops, grams
Rothamsted										
Control	7·6	100	0·702	0·049	1·6	100	1·686	0·025	9·2	0·074
Soil + 0·1 % CaO	15·9	209	0·803	0·117	1·3	79	1·785	0·021	17·2	0·138
Soil + 0·2 ,,	17·0	224	0·925	0·147	2·5	155	1·570	0·037	19·5	0·184
Soil + 0·3 ,,	22·9	300	1·021	0·217	3·1	190	1·643	0·048	26·0	0·265
Soil + 0·4 ,,	20·7	276	1·183	0·227	4·0	245	1·769	0·066	24·7	0·293
Soil + 0·5 ,,	15·1	199	1·802	0·253	7·1	435	1·778	0·119	22·2	0·372
Soil + 1·0 ,,	1·0	14	3·586	0·034	17·7	1086	3·206	0·531	18·7	0·565
Soil + 1·0 % $CaCO_3$	7·6	101	0·641	0·046	1·9	118	1·533	0·027	9·5	0·073
Millbrook										
Control	8·3	100	0·826	0·065	1·7	100	1·979	0·032	10·0	0·097
Soil + 0·1 % CaO	15·3	184	0·942	0·138	1·1	64	1·918	0·020	16·4	0·158
Soil + 0·2 ,,	19·1	230	1·235	0·225	2·9	171	1·744	0·049	22·0	0·274
Soil + 0·3 ,,	22·5	271	1·523	0·327	5·3	306	1·560	0·077	27·8	0·404
Soil + 0·5 ,,	7·1	85	3·678	0·272	15·3	890	2·036	0·295	22·4	0·567
Soil + 1·0 ,,	0·4	1·5	4·019	0·015	12·0	697	3·227	0·366	12·4	0·381
Soil + 1·0 % $CaCO_3$	9·5	114	0·831	0·074	1·1	64	1·802	0·018	10·6	0·092
Craibstone										
Control	28·1	100	0·761	0·202	1·2	100	2·338	0·026	29·3	0·228
Soil + 0·1 % CaO	27·0	96	0·680	0·172	1·9	154	1·835	0·032	28·9	0·204
Soil + 0·2 ,,	30·9	110	0·652	0·192	1·8	148	2·071	0·036	32·7	0·228
Soil + 0·3 ,,	32·6	116	0·754	0·231	2·6	210	2·048	0·049	35·2	0·280
Soil + 0·4 ,,	37·3	132	0·872	0·305	3·6	295	2·260	0·076	40·9	0·381
Soil + 0·5 ,,	37·9	135	1·014	0·360	4·4	362	2·100	0·087	42·3	0·447
Soil + 1·0 ,,	45·0	160	1·544	0·637	8·0	654	2·246	0·168	53·0	0·805
Soil + 1·0 % $CaCO_3$	33·2	118	0·762	0·237	3·8	309	1·965	0·069	37·0	0·306
Chelsea										
Control	34·7	100	1·242	0·405	2·9	100	1·855	0·050	37·6	0·455
Soil + 0·1 % CaO	34·2	98	1·192	0·384	3·5	122	1·783	0·057	37·7	0·441
Soil + 0·2 ,,	39·9	115	1·254	0·470	3·9	135	1·784	0·066	43·8	0·536
Soil + 0·3 ,,	39·1	112	1·398	0·509	3·8	133	1·812	0·064	42·9	0·573
Soil + 0·4 ,,	38·4	111	1·689	0·614	5·4	190	1·658	0·084	43·8	0·698
Soil + 0·5 ,,	36·4	105	1·989	0·694	6·7	234	1·722	0·108	43·1	0·802
Soil + 1·0 ,,	32·6	94	2·495	0·668	16·5	572	2·307	0·364	49·1	1·032
Soil + 1·0 % $CaCO_3$	33·3	96	1·061	0·338	3·5	122	1·659	0·054	36·8	0·392

indicated by the relatively short period during which nitrification is inhibited. The ammonia and nitrate figures show a steady increase with these doses, so that the crop depression appears to be a physiological effect on the plant rather than a shortage of food[1]. Throughout the period of growth of the first crop, the barley plants appeared very flaccid in character; this effect was also evident in the third crop which was rye.

The second crop, mustard, does not show this effect, but gives a steady rise with increasing lime applications. In general, there is no such increased nitrogen content in plants growing in these limed soils, as was observed with the Rothamsted and Millbrook soils.

SUMMARY.

Caustic lime is found to have two distinct effects on the soil:

1. A partial sterilisation effect,
2. A chemical action, decomposing some of the soil organic matter.

The amount of caustic lime necessary to induce specific changes in the flora and fauna of the soil depends very largely on the character of the soil. The light sandy Millbrook soil, poor in organic matter and in carbonate, reacted sharply with 0·2 to 0·3 per cent. caustic lime; the Rothamsted clay soil, poor in organic matter but rich in carbonate, was found to react to 0·3 to 0·4 per cent.; the acid Woburn soil required an amount between 0·5 and 1·0 per cent., as did also the rich Chelsea garden soil, which already contained carbonate; the Craibstone soil, with a high organic and a low carbonate content, failed to react even to applications of 1·0 per cent. caustic lime.

Each of these soils, as well as many others examined, appears to absorb directly a definite amount of caustic lime, and until these requirements are fully satisfied the partial sterilisation phenomena do not set in. These phenomena include a sudden initial decrease and subsequent increase in the numbers of bacteria, the extinction of the larger forms of protozoa and the inhibition of nitrate production. Lower doses than those required for partial sterilisation induce a temporary suspension of nitrification, and consequent accumulation of ammonia, for periods varying with the amount of lime and the character of each soil; they also lead to a temporary increase in numbers of

[1] In a parallel case of peaty soils Ritter (17) suggested that the injurious effect occasionally obtained on liming may be due to oxalic acid produced, and Densch (18) suggests a reduction of nitrates to nitrites.

bacteria capable of growing on gelatine plates, but these afterwards decrease until the level of the untreated soil is reached.

Caustic lime chemically breaks down some of the organic matter of the soil, as shown by the ammonia formed during periods when soil bacteria are quiescent; when, however, bacterial growth commences, there is a large increase in the rate of ammonia production.

The return in nitrogen, as ammonia and nitrate for each increment of lime applied, varies with the character and reaction of the soil and the carbonate content. On the average, and within a period of about 250 days, it amounted approximately to 1·0 per cent. by weight of the caustic lime applied. Carbonate gave less returns, apparently because of its relative inaction on soil organic matter.

The pot experiments show amounts of available nitrogen in the soils comparable with the amounts of ammonia and nitrate produced in the laboratory experiments. In some cases the amount of caustic lime applied was sufficiently large to check the growth of bacteria and to depress plant growth in the first crop[1], but in the case of the Chelsea soil the bacteria were active but plant growth was depressed, a phenomenon not yet satisfactorily explained.

As in other experiments, inhibition of nitrification resulting from applications of lime leads to a higher nitrogen content in the plants. This has been ascribed to the assimilation of nitrogen compounds other than nitrates, and, if occurring to any extent, involves an uneconomic utilisation of soil nitrogen. Where the amount of lime does not check nitrification, as in the case of the lighter dressings to the Craibstone soil, the nitrogen content of the plants is about the normal.

[1] A certain agreement exists between maximum yields of first crop with applications of caustic lime sufficient to induce partial sterilisation (*e.g.* Millbrook and Rothamsted soils).

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THE DETERMINATION OF SOIL CARBONATES.

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WITH the extension of soil survey work and an increasing recognition of the importance, from chemical, physical and biological considerations, of the presence of calcium carbonate in the soil, it has become necessary to be able to determine accurately and with comparative ease the amount of carbonate in any given soil. During the past few years the methods adopted for this purpose have varied considerably, partly on account of inaccuracies which were only too apparent in comparative work, and also because of the need for greater convenience in manipulation.

It is not proposed to review here the literature of the subject, since this has been admirably done elsewhere[1], but an arbitrary division of previous methods into two classes may be made. The first class comprises those methods in which the calcium carbonate in the soil is extracted by the aid of weak acids (carbonic, acetic, citric, etc.) and the amount of calcium estimated either gravimetrically after precipitation with oxalic acid or volumetrically by the determination of the free oxalic acid remaining, by means of permanganate. The second class contains all those methods where the carbonate is decomposed by the use of various mineral acids, and the amount of carbon dioxide evolved is (*a*) measured directly, (*b*) absorbed and determined either gravimetrically or volumetrically, or (*c*) estimated by the reduction of pressure on expanding into a vacuum.

It is obvious that in the first group of methods all soluble calcium salts are included in the determination as well as the carbonate, while

[1] W. H. MacIntyre and L. G. Willis. *Bulletin* 100, Agric. Exp. Stat. Tennessee.

in the second group other carbonates besides calcium are estimated. The second, however, gives the more useful information, and being more convenient and rapid in operation such methods have been used most extensively in routine soil analysis. During the past few years the tendency has been to limit as far as possible the degradation of organic carbon soil compounds with production of carbon dioxide and with accompanying high results. The two chief factors operating in this direction have been the use of comparatively strong acids, and the high temperatures at which the determination was carried out, or a combination of these two.

For the determination of small quantities of carbonate, Hall and Russell[1] proposed a method in which the soil was exposed to the action of sulphuric acid (1 : 1) in vacuo at room temperature, the carbon dioxide liberated being estimated by an increase in pressure in the apparatus. Amos[2] adopted the double titration method recommended by Brown and Escombe and collected the carbon dioxide in 4 per cent. sodium hydroxide solution contained in a Reiset tower, the carbonate being decomposed by boiling with 20 per cent. hydrochloric acid. Marr[3] modified this method by boiling at 50° C. under reduced pressure and using hydrochloric acid of 0·8—2·0 per cent. strength. He was able to show that when soils are boiled at 100° C. with weak acids or even water there occurs an appreciable evolution of carbon dioxide and that, with rich organic soils and strong acid, this amount may approximate to 0·6 per cent. of the air-dried soil.

During subsequent work in this laboratory, A. V. Campbell modified the method still further by employing 2 per cent. hydrochloric acid and carrying out the estimation at room temperature. Quite recently MacIntyre and Willis[4] found that when soils are digested with hydrochloric, sulphuric and phosphoric acids in dilutions of 1 : 5, 1 : 10, and 1 : 15, the latter acid induced the least formation of carbon dioxide from soils almost devoid of calcium carbonate, and upon these data suggest the use of phosphoric acid (1 : 15) at room temperature, the carbon dioxide being removed from the reaction flask by aspiration of air and collected in soda lime tubes. Aspiration for 30 minutes with constant agitation of the apparatus and the maintenance of a slight vacuum are stated to be the essentials of the method.

[1] Hall and Russell, *Trans. Chem. Soc.* 1902, **81**, 81.
[2] Amos, *Journ. Agric. Science*, 1905, **1**, 322.
[3] Marr, *ibid.* 1909, **3**, 155.
[4] MacIntyre and Willis, *loc. cit.*

In addition to the disadvantages, in the way of washing, which attach to all efficient gas-washing towers, the Reiset tower has additional ones in its high initial cost and in the number of ground-glass joints which, on agitation of the acid and soil component of the apparatus, tend to become displaced and lead to leakage. Moreover, a supply of water sufficient to work a filter pump is necessary.

In order to provide an alternative method for the determination of carbon dioxide the apparatus described below has been devised and is shown in Fig. 1.

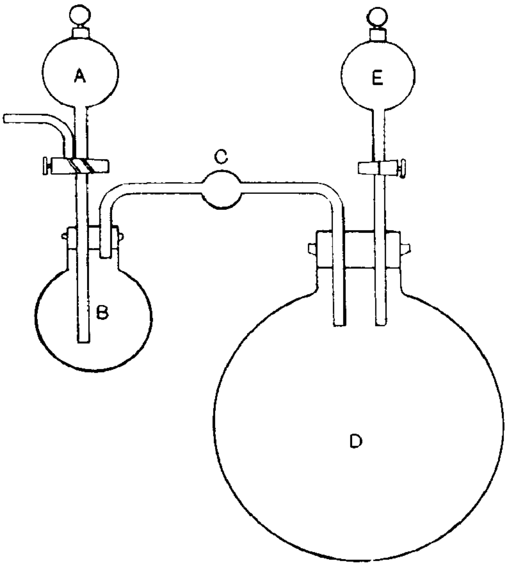

Fig. 1.

Two round-bottomed bolt-head flasks, *B* and *D*, of Jena glass, of 100 and 1000 c.c. capacity respectively, are fitted with double-bored rubber corks and connected by a stout bulb tube (*C*). Into the cork of flask *B* is fitted a special three-way glass stop-cock funnel (*A*) of 50—70 c.c. capacity, while an ordinary separating funnel (*E*)[1] is fitted into the neck of *D*. In making an estimation the soil, carbonate or solution is placed at the bottom of *B* and the corks fitted firmly into the necks of the two flasks. The funnel *A* is then opened to the air and 50 c.c. of 2 per cent. hydrochloric acid run in; the tap of the funnel *E* is closed, the stopper removed and 50 c.c.[2] of N/10 sodium hydroxide solution run in after which the stopper is replaced.

[1] The cylindrical form has been found preferable.

[2] If this should be insufficient a second 50 c.c. may be added during the experiment.

The apparatus is then connected with a good water- or hand-pump (capable of giving an internal pressure of 60—100 mm. of mercury) and evacuated. The tap of *A* is then closed and the sodium hydroxide allowed to run into *D* from *E*, taking care that one or two cubic centimetres of solution remain in *E* to absorb any trace of carbon dioxide which may leak through the tap from *D*. Acid is then run into *B* from *A* and the more vigorous stage of gas evolution allowed to pass before the apparatus is agitated. By attaching a number of such units to a small retort stand the whole may be shaken quite easily, or if one clamp only is used to support the apparatus by the neck of *D*, sufficient play will be afforded by the rubber cork of *D* to enable *B* to be shaken vigorously.

When all evolution of carbon dioxide has ceased (which usually occurs within 20 minutes) the side tube of *A* is connected with a gas-washing tower and air allowed to bubble into the apparatus with occasional shaking to remove any residual carbon dioxide in *B*. After about 20—60 minutes from the time air is passed in, it will be found that absorption is complete; the flask *D* may then be disconnected and the inside of funnel *E* and tube washed down with carbon dioxide-free water.

Either of two methods of titration may be adopted. The first is that of Hart, employed by Brown and Escombe[1], in which the free alkali is neutralised and the carbonate converted into bicarbonate, using phenol phthalein as indicator. As soon as all colour has disappeared methyl orange is added and the bicarbonate titrated against N/10 sulphuric acid: working with 10 grams of soil each cubic centimetre of acid being equal to 0·1 per cent. of carbonate in the soil. The disadvantage of this method lies in the fact that the colour change during the conversion of the last trace of carbonate to bicarbonate is extremely tardy and, indeed, it is often necessary to allow the flask to stand several minutes before completing titration, since upon this end-point depends the accuracy of the titration with methyl orange. Lunge[2] has shown that this method of estimation is sufficiently accurate for the determination of very little carbonate in the presence of much free alkali but not under reverse conditions. Further investigation[3] showed that by the addition of sodium chloride values more closely approaching to the real ones could be obtained, provided that the relation of sodium chloride

[1] Brown and Escombe, *Phil. Trans. Roy. Soc.* 1900, **193**, 289.

[2] Lunge, *Ztsch. angew. Chemie*, 1897, 41.

[3] Lunge and Lohöfer, *ibid.* 1901, 1125.

to sodium carbonate was at least 1·75 mol. of the former to 0·5 mol. of the latter.

A second method, which has been adopted by us since it gives a better end-point, consists of the addition of excess of barium chloride to remove carbonates from solution and then to titrate the free alkali alone with phenol phthalein if N/10 soda has been used for absorption. This, deducted from the reading for free alkali in the solution in a blank experiment, may be taken alone for calculation, or the barium carbonate precipitate may be further titrated against acid after the addition of methyl orange. It is advisable, however, to add the barium chloride solution only immediately before titration since, on prolonged standing, there is a tendency to the formation of larger aggregates with a consequent slowing down of the last stage of the titration with methyl orange. Even in this eventuality a slight excess of acid may be added and a back titration made with N/10 sodium hydroxide solution.

Appended are some of the experimental results obtained by the use of this apparatus.

Substance	Amount taken	Amount found
Calcium carbonate. (Kahlbaum)	0·0500 grm.	0·0510 grm.
,, ,,	0·1000 ,,	0·0990 ,,
,, ,,	0·1500 ,,	0·1487 ,,
,, ,,	0·2000 ,,	0·1997 ,,
,, ,,	0·4000 ,,	0·4007 ,,
Sodium carbonate	0·1924 ,,	0·1934 ,,
,, ,,	0·0935 ,,	0·0944 ,,
,, ,,	0·1871 ,,	0·1850 ,,
Harpenden Field Soil	10 grams	0·1065 ,,
,, ,, ,, + 0·200 grm. $CaCO_3$	,,	0·3060 ,,
Calcium carbonate		
Absorption period 20 min.	0·200 grm.	0·1990 ,,
,, ,, 40 ,,	,,	0·1995 ,,
,, ,, 60 ,,	,,	0·1990 ,,

THE SCOURING LANDS OF SOMERSET AND WARWICKSHIRE.

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A CONSIDERABLE area of pasture land in Mid-Somerset, known locally as "teart" land, and also a smaller area in Warwickshire, cause scouring in cattle at certain times of the year. These pastures are a source of loss to the farmers and, from time to time, have been the subject of investigation. In 1862 the late Dr J. A. Voelcker published a valuable report on the subject[1], and in the main the observations and results discussed in the present paper are consistent with the suggestions which he put forward. Details of more recent work are to be found in various reports in the *Journal of the Bath and West Society* from 1895 to 1904.

An account of the special peculiarities of the Somerset scouring lands, together with some preliminary discussion of the characteristics of the soils, has been given in a previous paper[2]; and hence a detailed description is unnecessary here. As however the existence of similar land outside the county of Somerset was not then known to the writer, it is necessary to remark that the scouring land in Warwickshire shows precisely the same phenomena under precisely the same conditions. The scouring is most troublesome in the autumn in mild, damp seasons, and affects the animals in exactly the same manner as in Somerset. The same plan is adopted in dealing with the difficulty, viz. heavy feeding with cotton cake and the removal of the animals to other land. The general character and the properties of the soils are exactly the same as those of the Somerset soils; but in Warwickshire there is not such

[1] *Journ. Bath and West Soc.*, Vol. x. 1862, p. 183.
[2] *Journ. Board of Agric.* Vol. XVII. 1910, p. 529.

sharp demarcation between sound and scouring land as in some parts in Somerset, and there do not appear to be any districts so bad as the worst districts in that county. Otherwise all the conditions are identical and it is interesting to note that the suggestive name—Starveall Farm —occurs in one or two places in both counties.

The three suggestions most frequently put forward to account for the phenomena of scouring are (1) a bad water supply, (2) the presence of one or more particular species of plants in "teart" pastures, and (3) the presence of a specific organism. It is easy to show that neither of the first two of these suggestions can be substantiated[1]. With regard to the third, it is true that at first glance there seems much evidence in favour of the view that the scouring is a definite disease due to a specific organism. All attempts to isolate a responsible organism have however proved useless[2]; and, indeed, a further examination of the facts renders this biological theory of the origin of scouring highly improbable.

In many places, "teart" and sound land occur intermingled in the closest possible manner, and yet infection never travels from a "teart" field to a neighbouring sound field even though only a ditch may separate the two; nor do cattle transferred from "teart" to sound pastures ever bring infection to healthy cattle they may come in contact with. Again, the degree of "teartness"—the extent to which it may cause scouring—differs greatly in passing from one field to another. It is true that, as will be seen later, the soil varies correspondingly: but, if a micro-organism of any kind is really responsible for the scouring, it will be necessary to imagine it affected by a slight change in the soil in a very remarkable manner.

Moreover, the symptoms do not suggest a specific disease at all. The severity of the attack appears to be dependent on the constitution and general health of each animal and may in nearly every case be checked by removal to sound land.

Type of Soil.

The most striking fact in connection with the scouring pastures is that they are all found to be situated on the same geological formation—the Lower Lias. Although the greater part of this formation in England generally is covered by drift or beds of sand and gravel, this is not the case in Mid-Somerset. The Lias soil there

[1] *loc. cit.*

[2] For some bacteriological details, see Newman, *Journ. Bath and West Soc.* 1897 and 1903.

has been formed *in situ* and is a characteristic sticky yellowish clay, extremely hard when dry. The subsoil is a still stiffer clay, often bluish in colour. In those parts of Warwickshire where similar scouring is met with (also on the Lower Lias formation), there is again not much evidence of drift; and, except in these two counties, so far as the writer is aware, such serious scouring pastures do not occur. It is, however, interesting to learn that in parts of Lincolnshire, notably the Vale of Belvoir, cattle do sometimes scour a good deal when first turned out in the spring or when grazing luxuriant aftermath, though the harm done is apparently never serious. Here also the Lower Lias formation is not obscured to any great extent by overlying drift soils.

In Somerset a large part of the districts in which the "teart" land is found is at a low elevation and is covered by an alluvial deposit, varying from a few inches to many feet in depth, and becoming peaty in some localities. Pastures on this alluvial land are invariably free from any tendency to cause scouring even though the typical Lower Lias clay may lie not far below the surface. The division between sound and "teart" land is in these districts very sharp and indicates accurately the boundary between Lias and Alluvium; and the same kind of thing may be met with at the junction between the Lias and other formations, *e.g.* the Inferior Oolite[1]. An examination of the surface soils of neighbouring "teart" and sound fields always shows a corresponding difference in appearance and "feel." In extreme cases where there is an alluvial deposit of great depth, the differences are very obvious: a black friable loose soil in the one case, and a heavy sticky clay in the other. But even where the soils are in most respects very similar, the surface of the sound fields is darker in colour (containing more organic matter), looser in texture, and especially after heavy rain or after prolonged drought has a different "feel" on walking over the ground. To whatever cause the difference in the nature of the top few inches of soil is due—whether to the deposition of a layer of alluvial soil or to some process of cultivation—the result is a diminution in the tendency to produce a scouring herbage. Evidently therefore the primary cause of "teartness" is closely associated in some way with the soils.

Chemical Analysis.

Chemical examination of the mineral constituents of the soils of "teart" pastures has not revealed anything that could account for the

[1] See *Journ. Board of Agric.* Vol. XVII. p. 537.

observed effects. There is no obvious peculiarity. Other workers have also failed to trace anything unusual in the chemical composition of these soils[1].

Soil Densities.

In order to investigate further the difference in texture between "teart" and sound land, determinations of the densities of some of the soils *in situ* have been made. These determinations have been confined to soils in Somerset, since in the Warwickshire scouring districts there is not a sufficiently sharp change from sound to scouring land to afford material for accurate comparison.

To obtain the soil density, small metal boxes (accurately 2″ square) with removable top and bottom were used; and by taking a number of samples, the average weight of 8 cubic inches of the surface soil *in situ* could be obtained.

The greatest possible care was taken to procure all the samples in precisely the same manner; and fortunately for this purpose all these soils are almost entirely free from stones.

In Table I the detailed figures are given for all the samples from two fields, which will give an idea of the degree of accuracy obtainable by this method; and Table II gives the figures for the densities in a number of cases. These have been calculated from unit weight of *dry* soil, the moisture in each sample being determined and allowed for. This method is to some extent conventional since it involves the assumption that the volume of the wet soil is equal to the volume of dry soil + the volume of the water, which is probably not strictly true. The results are, however, comparable and sufficiently accurate for the present purpose.

In spite of some irregularities, it will be seen that the difference in texture between "teart" and sound land is reflected in the figures for the densities; these show, sufficiently consistently, a definitely lower density for the surface soils of the sound fields.

The contrast between these types of soil was also well illustrated in one or two cases by their respective hygroscopic properties. The samples in one locality were taken on Nov. 9th, 1911, when some heavy but not continuous rain had fallen after the long drought of the summer, and the ground was not thoroughly soaked. The difference in apparent "wetness" between the soils of the "teart" and sound fields was so marked that it was difficult to believe that the same amount of rain had

[1] *Journn. Bath and West Soc.* 1862 and 1903.

fallen on both. The soil of the sound field seemed much dryer and was hard enough to make it difficult to get the samples, whilst the "teart" soils could be moulded with the fingers. Actually the percentage of

TABLE I.

Sample No.		Wt. 8 cub. in. wet soil in grams	Moisture per cent.	Wt. dry soil in grams
Sound Field	1	170	35·12	110·3
	2	173·5	35·97	111·1
	3	163	38·42	100·4
	4	171·5	36·13	109·5
	5	160	38·63	98·2
	6	168·5	36·34	107·3
				Mean = 106·1 grms.
Teart Field	[7	171	38·9	104·5]
	8	189	35·16	122·6
	9	203	32·48	137
	10	198	35·75	127·2
	11	185	34·36	121·4
	12	200	33·47	133
				*Mean = 128·2 grms.

* Neglecting Sample No. 7 as unrepresentative.

TABLE II.

Locality	Wt. dry soil in 8 cub. in. in grams		Density	
	Teart	Sound	Teart	Sound
Queen Camel	160·0	114·5	1·22	0·871
Kingsdon	123·3	100·9	0·94	0·769
Pylle	128·2	106·1	0·98	0·809
Podimore	134·7	103·5	1·03	0·789

Six samples were taken in each field and the mean figures are given.

moisture was distinctly *higher in the sound soil*, the mean figures for five samples in each field being:

Sound, 31·2 per cent. moisture,
Teart, 25·8 " " "

The sound field in this case has no surface deposit of alluvial soil; its history differs from that of the surrounding pastures (all more or less "teart") only in the fact that, years ago, it was drained by open "cuts." The effect of this surface drainage is the only possible explanation of its superiority.

Similarly, in another district, where the sound fields lie at a lower elevation, are often flooded in winter and are covered by a thin deposit of alluvium, the mean figures for moisture were: sound, 33·27 per cent.; teart, 31·8 per cent.; the sound soil being again *apparently* dryer than the other.

Mechanical Analyses.

The physical differences between sound and scouring soils might be due to a difference either in the proportions of the various sizes of the ultimate particles, or in the arrangement of the temporary aggregates and compound particles. In the former case, the results of mechanical analyses of the soils should provide the explanation.

In Table III the analyses of a number of pairs of soils from scouring districts in Somerset are given; and in Table IV the analyses of five soils

TABLE III*.

	Edgarley		Westhay		Kingsdon		Pylle		Bishopsworth	
	Teart	Sound	Teart	Sound	Teart	Sound	Teart	Sound	Teart	Sound
Gravel	—	—	1·06	0·07	—	—	0·5	0·2	0·47	—
Coarse sand	0·5	0·57	6·02	1·06	1·61	0·79	1·56	1·18	2·24	0·71
Fine sand	8·4	4·33	8·45	2·64	6·27	5·26	7·19	9·18	5·76	5·42
Silt	13·8	11·23	11·16	5·79	10·35	9·37	10·08	12·54	8·62	5·86
Fine silt	26·46	28·68	27·35	19·14	25·96	29·68	24·56	26·04	23·06	21·39
Clay	24·96	23·78	20·66	15·39	29·31	27·45	25·05	22·84	31·63	35·61
Moisture	8·78	9·34	6·53	12·78	5·94	7·00	8·04	7·40	5·55	12·0
Loss on ignition	14·53	19·84	15·59	40·97	17·11	19·00	16·17	16·30	16·46	16·69
Calcium carb.	0·22	0·17	2·02	0·28	2·41	0·05	4·69	1·74	1·31	0·35
	97·65	97·94	98·84	98·12	98·96	98·6	97·84	97·58	98·10	98·03

* "Sound" and "Teart" refer in each case to fields closely adjoining—at Edgarley separated only by a ditch or "rhyne."

from scouring districts in Warwickshire and of two from a neighbouring district where the Lias is drift-covered and where scouring is unknown. The figures for the soils from the two counties, though not unlike, differ

more than had been expected, in view of their close resemblance in appearance and general properties, the Warwickshire land containing on the average a higher proportion of the coarser particles, probably due to the admixture of some sand with the true Lias clay soil. The drift soils are, however, of a totally different character. On the other hand, all the Somerset examples, both the scouring soils and the neighbouring sound soils, are of the same type.

In view of these results, it is therefore evident that the observed differences in physical condition and texture cannot be accounted for by referring them to the ultimate mechanical compositions of the soils.

TABLE IV.

	North Warwickshire			South Warwickshire		Drift soils	
	I Broadwell Scouring	II Broadwell Scouring	III Broadwell Scouring but less than I and II	IV Chadsunt Scouring	V Kineton Scouring but less than IV	Non-scouring	Non-scouring
Gravel	1·13	0·2	0·39	—	—	0·44	0·89
Coarse sand	9·97	3·99	3·02	13·23	17·2	28·21	23·24
Fine sand	6·23	4·72	4·15	8·46	7·63	23·60	20·34
Silt	6·48	6·55	6·98	(7·1)	8·56	9·29	9·59
Fine silt	18·74	21·51	24·08	19·58	21·81	13·72	16·13
Clay	23·81	37·45	33·31	23·16	20·55	14·13	16·77
Moisture	5·22	6·59	5·89	6·16	6·39	2·04	2·33
Loss on ignition	12·25	10·39	14·87	12·84	15·92	6·63	9·02
Calcium carbonate	14·05	4·72	4·4	8·8	2·04	0·19	0·25
	97·88	96·12	97·09	99·33	100·1	98·25	98·56

These analyses have, however, brought out the fact that, in any pair of neighbouring fields there is, invariably, a higher percentage of organic matter in the soil with least tendency to cause scouring. This has been found to be generally true throughout the whole "teart" land area. Now the proportion of organic matter here unquestionably plays the most important part in the arrangement of the temporary aggregates and compound particles and hence to a great extent determines the texture of the soil.

Since, therefore, the only persistent difference observable between sound and "teart" soils lies in the textures, it is justifiable to consider *the*

special texture of the soil as a leading factor in determining "teartness." If this is correct, "teartness" would be expected to disappear when the soil texture is improved; and field experiments are needed to test the practicability of methods of improvements which suggest themselves if this view be accepted. There is, indeed, already a certain amount of evidence available which points in this direction. For example, "teart" pastures when ploughed up and resown after a few years only slowly become "teart" again. Then, the effect of *surface* drainage is well shown in the case discussed on p. 333; and similar but less striking results have been effected elsewhere, though "teart" pastures do not as a rule lie wet.

Consideration of all the facts seems to render inevitable the conclusion that the texture of the soil plays the determining *rôle* in the production of scouring herbage.

The question then arises as to how the soil texture can affect the physiological properties of the herbage in this manner. There must certainly be a difference in the proximate constituents of grass from sound and scouring fields, but chemical analysis has so far failed to bring to light any substance to which the scouring properties could be attributed. The expressed juice and both fresh and dried material have been examined; and the results of analyses of some typical samples are given in Tables V and VI.

Analyses of the Herbage.

TABLE V. *Percentage of Fresh Material.*

Locality		Moisture		Ash		Total Nitrogen		Protein Nitrogen		Fibre	
		Teart	Sound	Teart	Sound	Teart	Sound	Teart	Sound	Teart	Sound
Westhay		67·59	57·15	3·79	3·9	0·63	1·03	0·50	0·78	7·92	10·70
Kingsdon	Oct. 14	79·45	76·28	2·8	2·6	0·50	0·45	0·33	0·25	4·69	6·55
Kingsdon	Dec. 9*	72·3	68·37	2·93	3·15	0·69	0·57	0·43	0·49	7·15	8·55

* After frost.

It has been noticed that on comparing the herbage from "teart" and sound land in the same neighbourhood, the former appears to be less mature than the latter[1]—is softer and less fibrous. This is borne out

[1] This has been previously noted and discussed by Voelcker. For other analytical data in this connection see *Journ. Bath and West Soc.* 1862 and 1903.

by the figures quoted in Table V: the "teart" herbage contains more water and less fibre than that from sound land when samples taken on the same date are compared. It is in seasons when the grass is very lush and grows rapidly that scouring is worst, and such conditions might be expected to accentuate any tendency to produce a somewhat unripened growth.

TABLE VI. *Percentage of the Ash.*

	Calcium oxide		Magnesium oxide	
	Teart	Sound	Teart	Sound
Westhay	5·52	7·55	2·25	3·37
Kingsdon	9·90	12·24	2·42	3·91

The failure to detect anything abnormal in the chemical composition of the scouring grass is perhaps not surprising in view of the difficulty of getting a really representative sample of the fresh material—a difficulty which is enhanced in the present instance because there is no means of knowing how far the herbage from different parts of the same field may vary in scouring properties. The facts, however, all point to the conclusion that scouring is due to the physiological action of some constituent or constituents of the herbage which are not normally present but only occur under special soil (and weather) conditions; and further that the soil conditions are determined by the texture and can be removed when the texture is appropriately changed.

In conclusion the writer wishes to express his best thanks to Dr E. J. Russell for much valuable advice; and to Mr B. W. Bull, Agricultural Adviser to the Warwickshire County Council, for his kind help in connection with the investigation of the Warwickshire soils; and also to record that this work was made possible by substantial grants to the University of Bristol from the Agricultural Instruction Committee of the Somerset County Council and from the Development Fund Commissioners.

THE DISTRIBUTION OF THE OVERHEAD ELECTRICAL DISCHARGE EMPLOYED IN RECENT AGRICULTURAL EXPERIMENTS.

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Introduction.

For many years the effect of electricity upon the growth of plants has been a matter for speculation and experiment, but it is only in recent years that improvements in the methods of generating high tension electricity have permitted of well-controlled experiments upon the problem. In these experiments the high tension electricity is discharged from a system of thin insulated wires stretched over the crops. Since 1912, with the aid of a grant from the Development Fund through the Board of Agriculture, we have been studying this problem and it is the object of this paper to point out certain modifications which our experience suggests should be made in subsequent field experiments on this subject. We have been led to these conclusions by a long series of measurements carried out through the kindness of the Yorkshire Council for Agricultural Education, at the Manor Farm, Garforth, where a high tension discharge system has been installed. In this paper these measurements are only given in such detail as to justify the conclusions we wish to emphasize and very little space is given to the technical details of the apparatus used. If any other investigators would desire fuller details as to the methods of measurement we now employ in these experiments we should be glad to supply these further particulars upon application.

Distribution and Variation of Discharge.

Apparatus. High tension electricity at from 50,000 to 100,000 volts was generated by the Lodge Newman system of transformer, valves etc. that has been previously described[1] and was supplied to an insulated network of thin wires raised some fifteen feet from the ground.

The first point investigated was the distribution of the discharge from the network to the ground. Various methods were tried for this investigation, and in the end the plan adopted was the exploration of the air in the neighbourhood by an insulated candle flame electrode, held at a definite height, and connected with an Exner aluminium leaf electrometer with a range from about 50 to 800 volts. Close to the wires this electrometer had often to be replaced by a Braun electrometer with a range up to 4000 volts. With these instruments an examination was made of the electrical conditions under the wires and in their neighbourhood, at points where in previous experiments the "control" area had usually been placed.

Measurements. Conditions in Garforth were not very suitable for accurate measurement of potential distribution, because of the smoke laden air. When the wind blew from the direction of some collieries, S.E. of the experimental field, the atmospheric potential varied very greatly, the gradient varying from 50 to 1000 volts per metre. A close study of the atmospheric electrical conditions was therefore made before charging the network, and it was found that except with winds from the S.E. the electrical conditions were so constant as to admit of no confusion between normal atmospheric electrical effects and variations of potential due to discharge.

The procedure adopted was the following. After an examination of the atmospheric electrical conditions, the discharge was switched on to the network and measurements taken at a series of places outside the charged wires. At the same time the wind direction was noted and a rough determination made of its velocity by means of a portable anemometer. A very large number of such observations were made to determine the relation between the wind and the discharge and from these data reliable conclusions can be drawn as to the distribution of the discharge under different conditions.

A difficulty involved in determining this relation is that the potential obtained at any point varies so rapidly with slight alteration

[1] See J. H. Priestley, *Journal of the Board of Agriculture*, XVII. p. 1, XX. p. 582.

in wind velocity and direction. This is illustrated in Fig. 1, obtained on a day on which the normal atmospheric potential gradient did not vary more than from 120 to 150 volts per metre. In this figure the varying strength of the discharge is roughly expressed as the number of times per minute the electrometer obtained its full charge (of 800 volts) and discharged to earth.

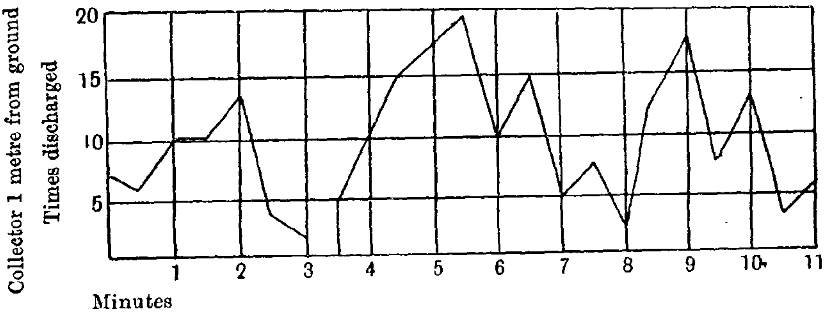


Fig. 1. Variations in strength of discharge at a point 70 yards distant from discharge network, illustrating the effect of wind variation.

It is clear then that any curve of distribution of the discharge with the wind will be only approximate, but such curves can be obtained, the method adopted being to ascertain the points where the potential, at a height of one metre from the ground, reaches the same value. These

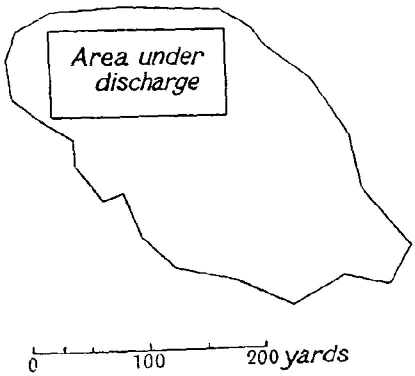


Fig. 2. Curve connecting points of equal potential (200 volts per metre) around discharge area, showing displacement of discharge by wind.

points will then give an irregular equi-potential curve which will indicate roughly the distribution of the discharge on that particular day. Many such curves have been obtained and they all show, as does

the example Fig. 2 reproduced, the great importance of winds in determining the distribution of the discharge. Fig. 2 was obtained upon a day with a light breeze with an average wind velocity of about 5 miles per hour.

Early in the season all measurements were made at a height of one metre from the ground, but as the crops advanced, reaching different heights at different points, the difference of potential was measured between two flame electrodes placed at different heights in the field. Tables I and II are examples of the values obtained, Table I being taken beneath the discharge wires, Table II outside them.

TABLE I. *Potential gradient volts per centimetre.*

Distance from ground	Successive measurements									
	1	2	3	4	5	6	7	8	9	10
95 cm.	105	165	170	135	85	110	90	70	120	95
75 ,,	100	135	145	110	140	80	90	110	60	110
55 ,,	75	110	140	60	80	55	60	95	45	75
45 ,,	85	65	50	65	40	75	85	100	95	80
35 ,,	65	40	45	55	60	55	60	65	70	75

TABLE II. *Potential gradient volts per centimetre.*

Distance from ground	Successive measurements									
	1	2	3	4	5	6	7	8	9	10
95 cm.	20	50	110	10	25	150	160	110	65	20
75 ,,	45	35	20	90	85	70	50	30	0	40
55 ,,	55	70	115	130	20	10	40	60	45	30

It will be seen that the values are higher but more uniform under the wires, though in either case it is difficult to draw conclusions as to the average potential gradient per centimetre. However on quiet days with no atmospheric disturbance the measurements show more agreement and allow us to suppose that the discharge, when independent of the wind and other disturbing factors, would occur in a uniform field except at points near the wires and near the plants.

The theoretical relation between potential gradient and distance from earth would then be expressed by the following curve, Fig. 3.

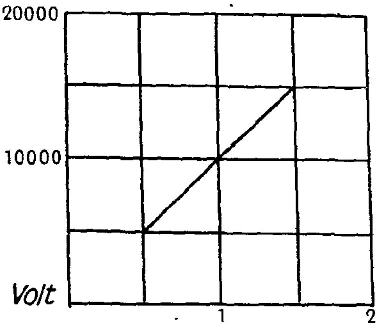


Fig. 3. Theoretical relation between potential gradient and distance from ground.

CURRENT DENSITY OF THE DISCHARGE.

Apparatus. After determining the potential gradient at various points in the experimental field attention was turned to the question of measuring the strength of the discharge by determinations of current density. For this purpose the Gerdien's aspirator was employed to determine the specific conductivity of the air, then, with the potential values determined as already described, the current density can readily be calculated[1].

Measurements. It has proved very difficult to analyse the resulting

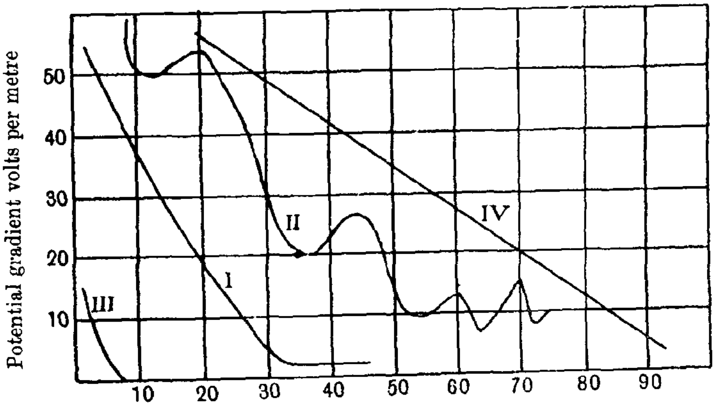


Fig. 4. Curves illustrating various types of distribution of the discharge against the wind.

[1] For details of instrument and calculations see Gerdien, *Physikal. Zeitschr.* 1903, IV. p. 632, and Ebert, *Phys. Zeitschr.* 1901, II. p. 662.

figures for current strength as the distribution of the discharge varies so very greatly with different atmospheric conditions. For the purpose of a provisional classification of the various types of distribution of discharge, a study of the distribution of discharge *against* the wind has proved of value and is made use of in distinguishing four types of discharge which are illustrated in the curves of distribution in Fig. 4.

The first of these types (I of Fig. 4) is the normal distribution in fine weather with the air free from serious contamination. In Fig. 5 is shown the distribution of the lines of equi-potential gradient under these conditions, with a wind velocity of from 3 to 4 miles an hour.

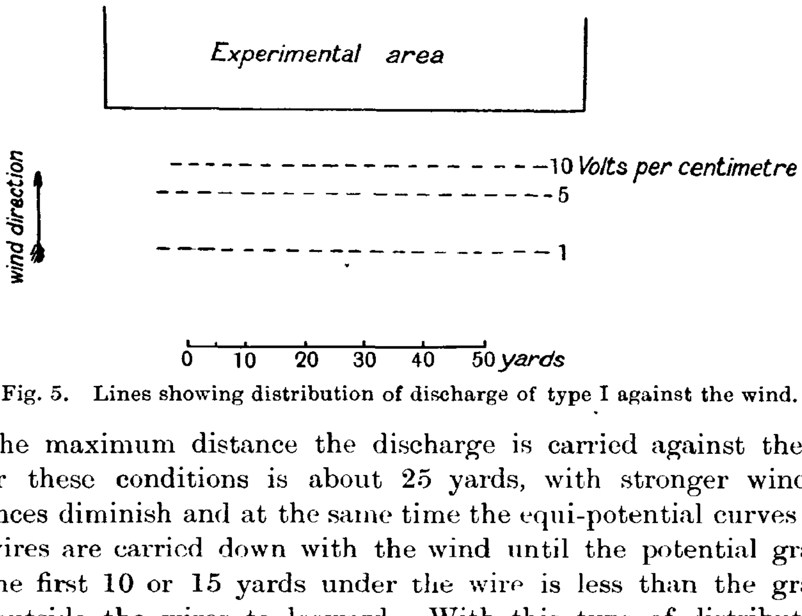


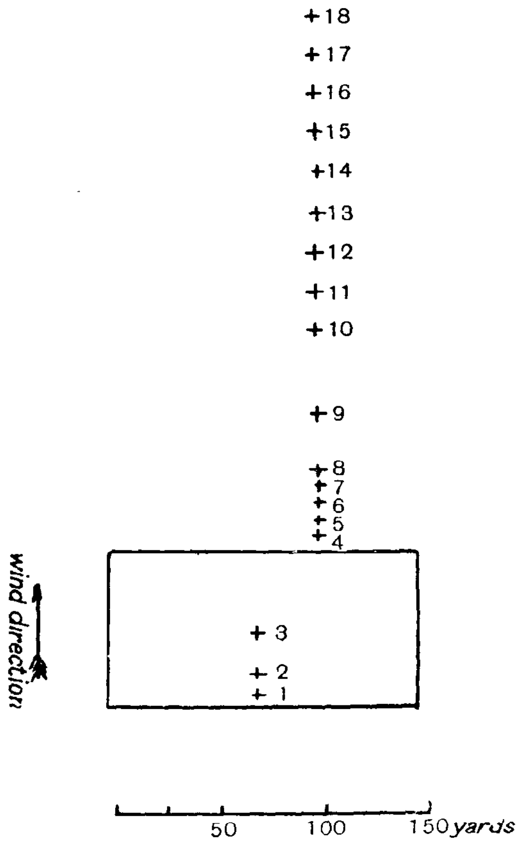
Fig. 5. Lines showing distribution of discharge of type I against the wind.

The maximum distance the discharge is carried against the wind under these conditions is about 25 yards, with stronger winds the distances diminish and at the same time the equi-potential curves under the wires are carried down with the wind until the potential gradient for the first 10 or 15 yards under the wire is less than the gradient just outside the wires to leeward. With this type of distribution of discharge the current strength at Garforth lay between 10^{-12} and 10^{-13} amp. (practical)/cm.^{2} With very strong winds it might fall as low as 10^{-14} amp./cm.^{2}

With the same general type of distribution measurements made at Lincluden give a higher value for the current. The difference is probably due to the fact that the discharge wires in the Garforth experiments were of bare galvanised iron and in the Lincluden experiments of cotton-covered wire. At Lincluden an average value for the current under comparable conditions would be 4×10^{-12} amp./cm.^{2}

Fig. 6 is a chart of the distribution of the discharge upon a very windy day. It will be noticed that the current *under* the wires on such

a day is small. The mean wind velocity during the measurement was 27 miles per hour. The average potential gradient under the wires (measured in the centre of the area) was 31 volts per cm. The gradients given are average values of 5 minute readings.



No	Volts per cm
1	18
2	22
3	31
4	31
5	22
6	23
7	21
8	17
9	12
10	13
11	11
12	8
13	10
14	6
15	9
16	3
17	5
18	2

Fig. 6. Distribution of the discharge on a very windy day. The potential gradient at the various stations is given in column of figures.

Fig. 7 and Table III illustrate the distribution of the discharge upon a quieter day, the average wind velocity being 11 miles per hour. The mean potential gradient and the mean conductivity are given for each place of observation. (Table III.) The observation stations are so chosen that a small variation in the wind direction will not materially affect the measurements.

The second type of distribution (II of Fig. 4) occurs in fine weather with smoke contamination present. The distance the discharge is carried

against the wind is not much greater than before but a definite low potential is never reached and it is difficult to tell when the effect of the discharge ceases. This type may not occur at experimental stations away from an industrial neighbourhood. The investigation of the strength of the discharge with this type of distribution is a much more complex

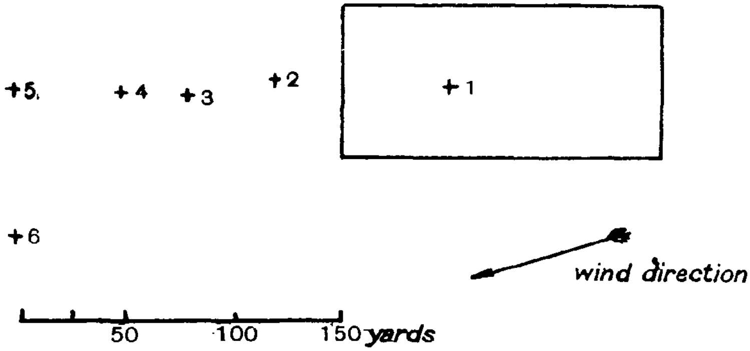


Fig. 7. Diagram to illustrate position of stations used for determination of figures given in Table III.

TABLE III.

Observation place	Potential gradient volts per cm.	Conductivity	Current Amp./cm.^{2}
1	91	0·008	8×10^{-13}
2	70	0·005	4×10^{-13}
3	58	0·002	2×10^{-14}
4	27	0·0007	2×10^{-14}
5	6	0·0004	3×10^{-15}
6	11	0·0006	7×10^{-15}

and intricate problem. The general feature is the great variability and the high value of the current density which may even reach the order of 10^{-12} amp./cm.^{2} under the wires. In other respects the distributions and variations of this type of discharge are governed by the same laws as the distributions and variations of the first type.

The third type (III of Fig. 4) is found on days on which condensation processes occur. It is quite distinct from the distribution of the discharge under rain when the phenomena approximate more nearly to the first type of distribution. This type gives the minimum values for the strength of the discharge, and its distribution outside the wires is

very limited. In Dumfries, with the cotton-covered discharge wires, it is a very frequent type of discharge, especially in damp weather. After some time the cotton covers dry and the discharge attains greater strength. The variations at Dumfries lie between 10^{-15} and 10^{-14} amp./cm.[2] At Garforth the figures are somewhat higher.

The fourth type (IV of Fig. 4) does not often occur at Garforth, but was found on several occasions at Lincluden, Dumfries, where an experiment station is at work. It occurs generally in quiet weather and shows the presence of very rapidly moving ions, probably of a radioactive nature. With these ions present the discharge makes its way much further against the wind. The maximum value of discharge with this type of distribution, which also is the maximum value of all current measurements under the wires, is $2 \cdot 3 \times 10^{-11}$ amp./cm.[2]

The general form of a distribution curve of any of these four types will be seen from Fig. 8. This curve apparently means that the distance the discharge is carried by the wind is proportional to the distance through which the wind has travelled under the wires.

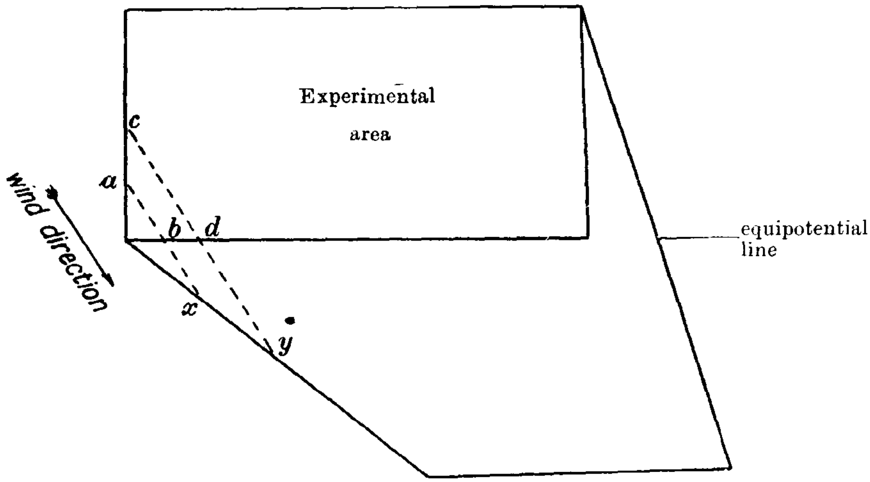


Fig. 8. Showing theoretical form of the distribution of the discharge, illustrated by the displacement of an equipotential line by the wind.

$$\frac{ab}{bx} = \frac{cd}{dy}.$$

Discussion.

It is clear that it is now possible to institute quantitative comparisons between the electrical conditions existing over so-called electrical

and control areas. Though the use of self-recording instruments will increase the accuracy of such comparisons, quantitative statements of the comparative electrical conditions must always remain approximate on account of the many variables involved. At the same time it is contended, that the means are now at hand to enable a sufficiently close comparison to be made for the purpose of gauging the effect of electrical discharge in improving crop yield.

Now that figures are available for the current density of the discharge in the neighbourhood of the discharge wires, it is interesting to compare these data with those given for normal atmospheric conditions. Under fine weather conditions the strength of the vertical current in the atmosphere is of the order 10^{-16} amp./cm.^{2} This figure does not vary very much in different parts of the world; the potential gradient is positive and of the order 1 volt per cm. More than 75 % of the rain is positively charged and the current densities are of the order 10^{-16} to 10^{-15} amp./cm.^{2} In thunderstorms the current densities considerably increase, from 10^{-15} amp./cm.^{2} to 10^{-13} amp./cm.^{2} and even to strengths of 10^{-12} amp./cm.^{2} for shorter periods.

Measurements of the atmospheric current, carried out in connection with this investigation, agree with these figures. In an industrial district such as Leeds the conditions are of course more complex than in a rural district like Dumfries. The average value for the normal current density at Garforth, arrived at as the mean of numerous measurements of the atmospheric current, is approximately 5×10^{-16} amp./cm.^{2}

As one result of this investigation we have to face the fact that the control and experimental areas in large scale field trials only differ from one another quantitatively, and that the success of an investigation into the effect of overhead discharge upon crop production will depend in part upon the ratio between the average current densities on the electrified and control areas[1]. If the current from the discharge were limited to the "electrified area" of the field we could estimate the ratio as follows:

$$\frac{\text{current density electrified area}}{\text{current density control area}} = \frac{10^{-12}}{10^{-16}} = \frac{10{,}000}{1}.$$

On the other hand when the electrical and control areas lie close together, as they have done in all experiments so far, there will be

[1] It must not be forgotten of course that the distribution of the electric *field* beneath the discharge wires will be much more restricted and constant. At the present stage in the general investigation the relative importance to be attached to the effect of the static field or of the current of discharge, is far from clear.

many days when the direction and strength of the wind together with other factors reduce this ratio to the order of $\frac{10}{1}$.

The great experimental difficulty lies in the fact that this ratio is so very variable, and we can only hope to get more definite data during future seasons by the use of self-recording instruments. At Dumfries during 1913, with the Exner electrometer and the methods described in this paper, Miss Dudgeon has carried out careful series of measurements which enable some idea to be obtained of the quantitative differences between the experimental and control areas. But even if it is possible to determine this ratio, this will not be sufficient for the purpose of large scale field trials. It is necessary to ensure that under all weather conditions the ratio between the two areas shall be as nearly as possible $\frac{10{,}000}{1}$ and as seldom as possible fall to $\frac{10}{1}$.

Considerations of soil and exposure, which must be alike in both areas, render it difficult to separate the areas, and therefore the experimental solution of the problem has been attempted along other lines. We have tried the effect of wire netting raised between the electrified and control areas and for part of the summer the two areas have been separated by such a network, ten feet in height and of half inch mesh. A decrease in the charge on the control area was thus obtained, but it was not sufficient. It will be necessary to raise the height of this screen and lower that of the discharging network (at present at a height of about fifteen feet). This will be done in 1914 at Dumfries, and Mr Low, who is conducting the field trials in progress at Balmakewan, is also arranging to place a wire screen between the control and electrified areas, the screen to be higher than the discharging network, and if possible to be kept charged to a relatively low negative potential.

In the Dumfries experiments during 1913, a plot of one tenth of an acre, situated some seventy yards away from the discharge network, was entirely enclosed by a wire cage of half inch mesh netting, six feet high. Even this was not sufficient to keep it completely screened from the electrical effect. Measurements taken upon a day when the wind was blowing from the electrical field gave an average content of 10^5 ions per cubic metre. Under the discharge wires, the average value for the content of ions per cubic metre was 10^9. Such closed wire cages are in any case unsuitable controls as several other factors associated with the plants' growth are altered under them, especially perhaps the moisture conditions.

It will be seen from this discussion of the subject that our attempt

to make the comparison between electrified and control areas quantitative in character and free from empiricism, has shown the problem underlying the technique of these field trials to be more complex than was anticipated.

However with a careful employment of suitable methods it should be possible to express with sufficient definiteness the comparative electrical conditions between these two areas. It should then be possible, especially as further data arrive from the laboratory, to ascertain whether the increase in crop production obtained from this treatment can be great enough to justify its employment on a practical scale.

Summary.

1. It is shown that the strength of the discharge from an overhead wire network at a high potential is a variable quantity depending on the mobility of the carriers of the electricity and on the velocity of the wind. Attention has been drawn to the presence of radio-active disintegration products which may possibly be a complicating factor in the effect of the discharge upon plant growth.

2. The maximum current density of the discharge in some experimental stations, where the apparatus is constructed after the pattern of the Agricultural Electric Discharge Co., is of the order 10^{-11} amp./cm.^{2}

3. Measurements of potential gradient and of current density agree in showing that the effect of the discharge is not limited to the area under the wires. This is of importance as control and electrified areas have usually been placed close together in field experiments. Some account is given of the distribution of the discharge under various weather conditions.

4. Methods are discussed by which the control area may perhaps be kept under more normal electrical conditions in spite of the proximity of the overhead discharge wires. The results of some of the tentative efforts in this direction which were made at Dumfries during 1913 are briefly discussed.

"QUALITY" IN WOOL.

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IN the year 1909 an experiment was started by Professor Wood to determine the mode of Inheritance of Wool and Mutton characters in Sheep. Two Merino Rams, sent to him by Mr Harper from Australia, were crossed with twenty pedigree Shropshire Ewes. The wool from these animals was sent to Professor Barker at the Bradford Technical College, and was there "sorted" in the commercial fashion adopted in Bradford for wool of its class. During the course of the experiment it became obvious that the commercial qualities were dependent upon a complex of characters, each of which was possibly independent in its inheritance. Consequently it was decided to attempt an analysis of the factors responsible for these characters.

Nathusius[1], Bohm[2], Konigsbom[3], Bowman[4], McMutrie[5] and others have shown that "quality" depends upon several factors among which are the following:

(1) Fineness of Fibre as measured by the average diameter.
(2) Number of Crimps or Waves per unit length of the fibre.
(3) Length.
(4) Lustre.

Owing to the fact that the wool yielded by this experiment was of a different class from that used by other investigators and also owing to the fact that it was necessary to determine the degree of accuracy with which individual sheep could be classed by the method employed, it was determined to repeat the work of these writers, and to subject the results thus obtained to a statistical analysis. In the course of the investigation over 700 slides were prepared and about 30,000 measurements made.

The present paper deals in particular with the relation between "Fineness of Fibre" and the Commercial Grading of the wool. The other wool characters are only considered in so far as they affect this relationship.

Methods.

(a) Methods of sorting and sampling.

At shearing, samples were taken from both of the shoulders, the neck, the britch, and the belly, an attempt being made to take the samples from similar positions on every sheep. The bulk of the wool was then sent to Bradford where it was sorted by a skilled sorter. A fleece was, in the course of this sorting, divided up into its various qualities, the weight of every class being taken.

The classes, into which the wool dealt with in this paper was sorted, were indicated by the numbers 64's, 60's, 58's, 56's, 54's, 50's, 44's, and a class for odd bits called 40's. These numbers, which are those employed in the Bradford trade, are based upon the spinning capacity of a given weight of wool. A fall in the numbers represents a fall in quality; thus 64's is the highest quality and 40's the lowest.

In addition to the samples taken from the sheep at shearing time, representative samples of the "qualities" were picked out for us by the sorter.

(b) Methods employed in the investigation of the samples thus obtained.

(1) Mounting.

The microscopic investigation of the "fineness" of the fibres, as determined by the average diameter, was based upon the method used by McMutrie ([5] pp. 47, 48). Four "sub-samples," A, B, C, D, were taken from each sample. From each sub-sample three portions were cut off, one at the tip end (T_1), another from the middle (T_2), and a third from the basal end (T_3).

Each portion was placed on a glass slide, and the fibres were arranged as parallel as possible by gently combing with dissecting needles[1]. Canada balsam was then added, and the slide placed on a hot plate until the balsam began to boil. The slide was then removed, a cover slip put on it, and pressed into place. By this means a permanent

[1] Incidentally a considerable amount of dirt was removed from the fibres during this combing process.

slide was obtained, and at the same time the fibres were cleaned by the action of the hot balsam.

The slides were labelled AT_1, AT_2, AT_3, BT_1, etc., to indicate the sub-samples they were made from and also the portions of those sub-samples.

(2) *Measurements.*

The measurements were made with a micrometer eye-piece, the microscope being so adjusted that each division of the scale represented $\frac{1}{8000}$ of an inch. The figures in this paper are, unless otherwise stated, based upon a unit of measurement equal to one eye-piece-scale division. Twenty readings were made on every slide by each of us. In order to avoid, as far as possible, the measuring of one fibre more than once, only those fibres were measured which lay approximately at right angles to the direction in which the slide was made to move across the field of vision. The fibres were measured in groups of four, these groups being uniformly distributed over the slide.

Such measurements have been made of the two shoulders of each of the F_2 sheep, and similar though not so complete measurements were made of the F_1 sheep, and of the approved samples of the "qualities."

In Tables I to V are given the figures obtained from these measurements[1].

The following symbols will be used throughout this paper to indicate the various results obtained from these measurements:

M_{40} = Mean of the 40 measurements made for each sub-sample.
M_X or M_Y = „ „ „ 160 „ „ „ „ shoulder.
M_P or M_E = „ „ „ 80 „ „ by each observer from shoulder.
M_{X+Y} = „ „ „ 320 „ „ on both shoulders.
σ_{160} = Standard deviation of the 160 measurements of one shoulder.
S.E.$_{160}$ = Standard error of average of 160 measurements of one shoulder.
P.E. = Probable error.

In dealing with the data given in the tables various considerations arise. It is proposed to consider in this paper the following points:

(*a*) The accuracy with which the average diameter obtained from the measurements of the four sub-samples of one shoulder may be taken as a true average of all the fibres in those sub-samples.

[1] The calculations involved were made with the help of a Burroughs adding machine kindly lent us for this purpose by Mr G. Udny Yule.

(*b*) The accuracy with which the average of the sub-samples represents the average of the whole shoulder sample from which these sub-samples came.

(*c*) The accuracy with which the shoulder samples may be taken for comparative purposes as representative of the whole shoulder.

(*d*) The personal equation in measurement.

(*e*) The relation between the figures obtained for the average diameter of the samples and the commercial quality of the wool as judged by the sorting at Bradford.

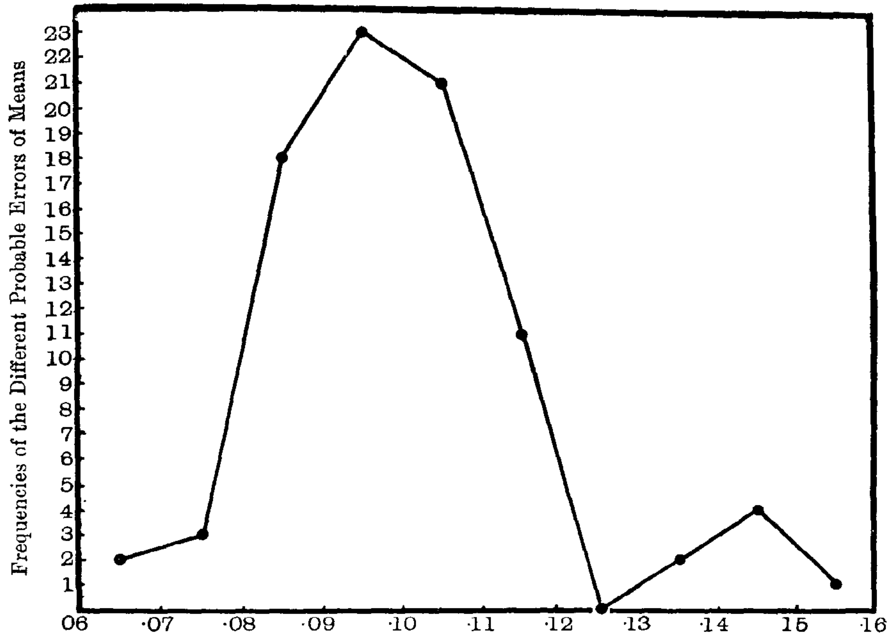
These points will be dealt with separately, although, as will be seen, they are connected *inter se.*

(*a*) *The accuracy with which the average diameter of the fibres measured in the four sub-samples of one shoulder may be taken as a true average of all the fibres in those sub-samples.*

As all previous investigators have shown, the range of variation in the diameters of individual fibres from the same lock is large. This variability is due in part to the fact that the fibres are not cylindrical and consequently have more than one diameter size, and partly to the differences of one fibre from another. Hence it is necessary to have some test for the accuracy of the average obtained. In order to obtain this test, the standard error of the average of every set of four sub-samples has been calculated. It was found that the standard errors thus obtained showed a considerable range of variation, and that they were to some extent correlated with the sizes of the averages; in other words, the variation in the coefficient of variation $\left(\frac{\sigma}{M}\right)$ was of less amplitude than that in the standard deviations, a result possibly due in part to the increasing differences between different diameters of the same non-cylindrical fibre as the size of the fibre is increased. From the standard errors, the probable errors of the various averages were calculated. The magnitude and distribution of the probable errors are given in Fig. 1. The amplitude of the variation of the probable errors is given by the range ·064—·154 or, as percentages of the averages concerned, 1 °/₀ to 1·6 °/₀. Thus, if we take even the largest magnitude of the probable error we can say that the odds are 20—1, that the average of 160 measurements is correct to within 5 °/₀, as this represents a range of three times the probable error.

(*b*) *The accuracy with which the average of the four sub-samples represents the average of the whole shoulder sample from which these sub-samples came.*

This point has been investigated by two methods. First by considering the range of the differences between the averages of any two



Magnitudes of Probable Errors of Means.
Class Interval = ·01.

Fig. 1.

sub-samples from the same shoulder. As has been stated, the averages of each sub-sample depend upon forty measurements, and there were four sub-samples, *A*, *B*, *C*, *D*, from each shoulder sample.

In Fig. 2 is shown the result of plotting the differences between sub-samples *A* and *B*, and between *C* and *D*.

The ordinate which divides this curve into two parts of equal area runs through the point 0.27 on the axis of the abscissae (the median). Hence half the differences between the two sub-samples of one shoulder are less than 0·27 units—a figure which is small compared with the averages concerned (about 3 to 4 °/₀ of the average). Consequently it can be stated that the average obtained by 160 measurements of the four sub-samples gives a good approximation to the average of the whole shoulder sample. This conclusion may be illustrated by considering the data furnished by the averages of the sub-samples in another way. Four cases showing exceptional fluctuation in these averages were taken,

and their standard deviations and probable errors of their averages were calculated.

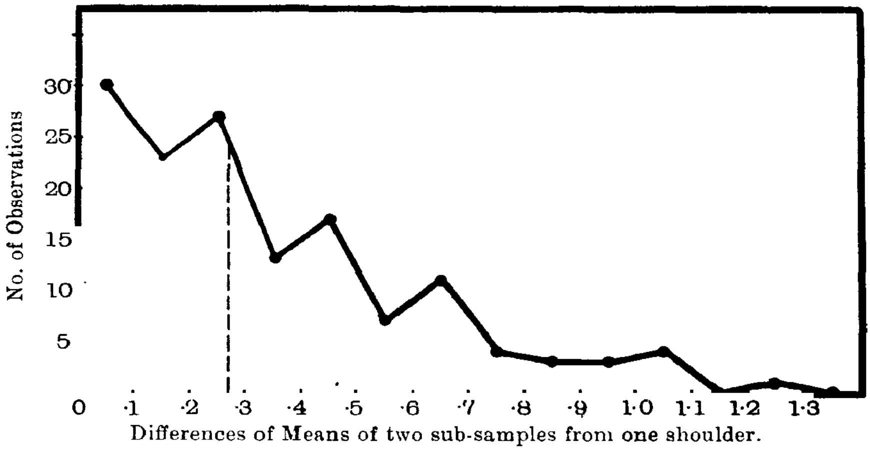


Fig. 2.

The results are shown in Table I.

TABLE I.

No. of Sheep	Sub-sample average	Average of 4 sub-samples	σ_4	Prob. Error of Average
F_2 ♀ 64 XT_1	7·83 7·98 9·10 8·80	8·43	·536	·180
F_2 ♀ 64 XT_2	6·78 8·10 8·30 7·78	7·74	·585	·197
F_2 ♀ 54 XT_1	7·45 7·95 7·90 8·85	8·04	·508	·171
F_2 ♂ 59 XT_2	8·95 8·28 6·80 7·90	7·98	·780	·263

Data given in this table show that the highest standard deviation observed is 0·780 giving a P.E. of 0·263, that is a P.E. of little more than 3 °/。 of the average. In other words, we can rely with fair safety,

even in cases showing the greatest variability, upon the average obtained from the sub-samples representing the whole shoulder average to within 10 °/₀. In the great majority of cases it will be correct to within 5 °/₀. These calculations (Table I) have been made merely for purposes of illustration and it is, of course, quite recognised that a standard deviation based on four magnitudes is not very trustworthy. The cases are, however, as stated above, selected for the exceptional divergences shown by the means, hence the standard deviations and the probable errors of the averages are larger than most cases.

As this point is one of considerable importance a further test was devised. The sample from shoulder *X* of F_2 ♀ 64 was taken as showing, as has been seen above, a high variability. A sub-sample was taken from every lock in the shoulder sample—20 in all (*E*—*X*). The customary twenty measurements were then made by each observer for every slide. Consequently 800 measurements were made in all.

The averages of these measurements in groups of four sub-samples, that is the averages of 160 measurements, are given in the following table, together with the average obtained by the 160 original measurements.

TABLE II.

Original Av.	Averages 800 new measurements in groups of 4				
M_{160}	*E*—*H*	*I*—*L*	*M*—*P*	*Q*—*T*	*U*—*X*
8·43	8·56	8·42	8·52	8·53	8·69

The greatest deviation is thus seen to be $8{\cdot}69 - 8{\cdot}42 = {\cdot}27$ or approximately 3 °/₀.

Taken as a whole the 800 measurements give a distribution for which

$$M = 8{\cdot}55,$$
$$\sigma = 1{\cdot}93.$$

The standard error for the mean of 160 measurements, that is for the number normally taken to obtain the average, is given by

$$\text{S.E.} = \frac{1{\cdot}93}{\sqrt{160}} = {\cdot}152$$ and the P.E. of the Av. of 160 measurements = ·103[1].

It is of interest to compare this result with the first test as to the representativeness of the average, that is with the test based upon the differences of the sub-samples for every shoulder.

The standard error for each of the twenty sub-samples is found from the equation

$$\text{Standard error of average of 40 measurements} = \text{S.E.}_{40} = \frac{\sigma_{800}}{\sqrt{40}}.$$

[1] The P.E. determined for the average of the original four sub-samples = ·101.

The probable error of the difference between the means of two sub-samples drawn from the main bulk is given by

$$\text{P.E. of Diff.} = \left[\sigma^2_{800}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)\right]^{\frac{1}{2}} \times (\cdot 6745).$$

Hence in the above case

P.E. of Differences of Averages of Sub-samples:—

$$= \cdot 6745\left[\frac{(1\cdot 93)^2}{40}+\frac{(1\cdot 93)^2}{40}\right]^{\frac{1}{2}}$$
$$= 0\cdot 291.$$

The first method showed that the corresponding magnitude—or the median difference—was 0·27. There is in fact a very close agreement between the results obtained from the two methods[1].

(c) *The accuracy with which the shoulder samples may be taken for comparative purposes as representative of the whole shoulder.*

The material available was not of such a nature as allowed a direct test to be made as to how closely the average of the shoulder samples represented the average of all the fibres from the shoulder area. But as each shoulder sample was taken as far as possible from similar points on the shoulder of each sheep, it seems sufficient for comparative purposes to investigate the errors likely to arise in the averages owing to small differences in the positions on the shoulder from which the samples came. This has been done by comparing the averages of the shoulder samples from the same sheep, a comparison which should give the maximum disturbances in the averages from this source. That this conclusion is a fair one is obvious when we consider that the differences between the shoulder samples of the same sheep will tend to be exaggerated by any lack of symmetry in the arrangement of the fleece.

The comparison between the shoulders was made by first finding the standard error of the difference in every case from the formula:

$$\epsilon_{XY} = \frac{\sigma_X^2}{n} + \frac{\sigma_Y^2}{n}.$$

The ratio between the difference of the averages for each shoulder to the standard error of this difference was then found. In the following diagram the result of plotting this ratio against the number of individuals is shown, *i.e.* the abscissae in the figure are the values of $(M_X - M_Y)/\epsilon_{XY}$ and the ordinates are the numbers of cases in which these different values were observed.

[1] It is interesting to note that the P.E. for the differences between the sub-samples declared from the 800 measurements of T_1 of F_2 ♀ 64 is slightly higher than that obtained by method (1), a result to be expected as F_2 ♀ 64 was chosen owing to its high variability.

From this diagram it is seen that 85 °/₀ of the differences between the shoulders are less than 1·5 times the standard error of the differences, and in 90 °/₀ of the cases less than twice the standard error[1].

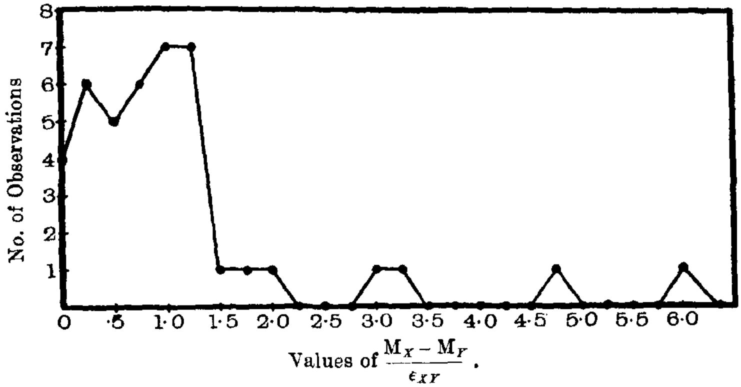


Fig. 3.

In comparing two sheep, or in comparing one sheep with the averages obtained from the Bradford quality samples, the average of both shoulders is taken. Consequently any error of the type considered under this heading is greatly reduced. Hence if the averages of the shoulder samples of two sheep differ by more than three times the standard error of their difference we may feel satisfied that this difference is real, and not due to any small variations in the position on the individual sheep from which the shoulder sample was taken.

(*d*) *Personal equation.*

As regards the facts discussed in this paper the question of the personal equation in measurement is not of vital importance, as all the results are comparative and in all important cases based on data obtained by averaging the measurements of the two observers. But in order to obtain a test by which the results of independent investigators can be compared it was decided to discuss the matter here.

The mean difference ·116 unit is small compared with the value about which the means concerned group themselves, only, in fact, approximately 1·4 °/₀ of that value. No difference (M_{160} 1st observer − M_{160} 2nd observer) exceeds 1·2 units (14 °/₀ of the general mean) and it is clear from the figure that in the great majority of the cases the difference is less than 5 units (*i.e.* 7 °/₀ of the general mean).

[1] The two outstanding results come from the T_1 and T_3 of the same sheep, namely F_2 ♂ 61. The disparity is possibly due to some error, and it is proposed to investigate the matter further with wool obtained from the fresh clip this year (1914).

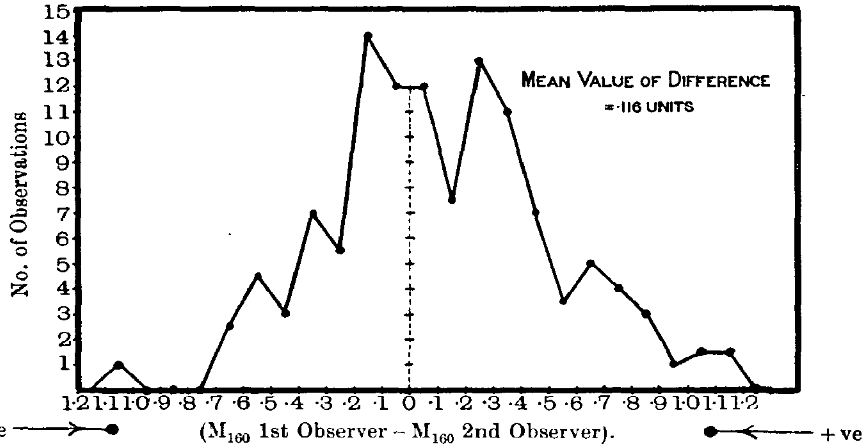


Fig. 4.

(*e*) *The relation between the figures obtained for the average diameter of the samples and the commercial quality of the wool as judged by the sorting at Bradford.*

As has been stated above, samples were taken at Bradford from each of the classes into which the wool was sorted. Eight sub-samples *A*—*H* were taken from each quality, a number equal to that taken from each shoulder of the individual sheep. The measurements were made as before.

In Table III are given the results of these measurements.

Leaving out of account the 40's which was made up of odd lots of various lengths and therefore is not strictly comparable with the other lots, it is seen that there is a general tendency to increasing fineness with rising quality, the correlation between the two being most obvious when fineness is measured by the average $\frac{T_1 + T_2}{2}$.

TABLE III.

Quality	T_1 Average Diam.		T_2 Average Diam.		T_3 Average Diam.		$\frac{T_1+T_2+T_3}{3}$	$\frac{T_1+T_3}{2}$
	M	σ	M	σ	M	σ	M	M
60's	8·11	*1·47*	8·11	*1·52*	5·41	*1·25*	7·21	6·76
58's	7·68	*1·88*	8·03	*3·19*	6·18	*1·59*	7·30	6·93
56's	8·28	*1·92*	8·28	*1·84*	7·16	*1·70*	7·91	7·72
54's	9·44	*2·35*	9·03	*2·26*	6·81	*1·86*	8·43	8·13
50's	8·93	*2·19*	8·83	*2·21*	6·92	*2·45*	8·23	7·93
44's	9·61	*3·51*	8·07	*2·71*	6·94	*2·87*	8·21	8·28
40's	8·36	*2·11*	8·18	*2·37*	6·18	*1·92*	7·57	7·52

But the correlation is by no means uniform whatever measure be taken for the fineness. Consequently it is of interest to consider these results in relation to observations made when watching the sorter at work. It was noticed that the sorting was largely based upon an examination of the base of the fibres, that is, upon that part of the fibres whose fineness is given by average T_3, but that the presence of any obvious coarse fibres at the tip of the lock was sufficient to put the wool in a low class. Allowance was no doubt made also for the waviness of the wool and for other characters. The possible modifying effect of these factors on commercial quality will be discussed later in greater detail when considering the relation between the average diameter of the shoulder samples of the fleeces and the highest quality assigned by the sorter to those fleeces.

In connection with the fact that "quality" determinations are largely based upon an examination of T_3 it is of interest to note that the fleeces under consideration are abnormal in that most of them have the T_3 average much lower than the T_1 average. McMutrie found that the reverse relationship was the most common, and we have found that some Shropshire shoulder samples sent us by Mr Cooper behave in the same way as McMutrie found to be the case. The smallness of T_3 in our fleeces may be in part genetic, but for the greater part it is pathological. The sheep from which the wool came had suffered from a severe attack of the "fourth stomach worm" (*Strongylus* species)[1], and the wool had been in some cases greatly affected; in certain fleeces to such an extent that a distinct line of weakness was apparent. Measurements showed that this line of weakness was closely connected with a fall in average diameter of the fibres. This is clearly seen in the case of F_1 ♀ 11. Four samples—*A*, *B*, *C*, *D*—were taken from the wool of this ewe. At intervals along the fibres of these samples readings of diameter were taken, the points of measurements on any one fibre being:

(*a*) At a distance of 2 cms. in the direction of the tip from the line of weakness.
(*b*) Immediately above the line.
(*c*) Immediately below the line.
(*d*) At a distance of 2 cms. in the direction of the base from the line of weakness.

[1] The T_3 average is the one to be affected as the sheep suffered most towards the end of the period for wool growth, *i.e.* in late winter, and throughout the spring (shearing was towards the beginning of June).

Table IV contains the results of these measurements, each number in the first four columns of the table being a mean of 20 measurements.

TABLE IV.

	A	*B*	*C*	*D*	Average
(*a*)	7·30	8·25	8·10	8·65	8·08
(*b*)	6·15	7·20	5·40	5·40	6·04
(*c*)	5·80	5·75	6·25	6·35	6·04
(*d*)	6·60	8·10	7·35	7·65	7·43

Clearly, therefore, the pathological decrease in diameter is approximately represented by $\left[\frac{(8{\cdot}08 + 7{\cdot}43)}{2} - 6{\cdot}04\right] = 1{\cdot}71$ units, *i.e.* about 22 °/$_\circ$ of the average diameter of the normal part of the fibre.

This pathological effect no doubt accounts for the fact that although the average T_1 diameter of the 60's quality is comparatively high (see Table III), the wool assigned to that quality seems to be very fine owing to the abnormally low T_3. In other words, wool of a comparatively low genetic quality tends to be classed as a comparatively high commercial quality owing to the pathological effect produced in the wool by the *Strongylus* attack. This is not to be taken as an argument for infecting one's sheep with disease because although the "quality" of the wool might be raised, the price paid for it would be decreased owing to the ease with which such wool breaks in the manufacturing process. It is rather to be taken as an argument for judging wool from a genetic point of view on the T_1 average, as the wool in this part of the fleece comes from the sheep when it is likely to be in its best health; most sheep diseases being commonly more virulent in late autumn, winter, and early spring than in the summer.

Further evidence in support of the contention that fineness as judged by average T_1 diameter is of fundamental importance from a genetic point of view, in estimating the quality of a fleece, is gained by considering the relation between this average for the shoulder diameter of the sheep and the highest quality assigned by the sorter to this fleece[1].

In the following figure the highest quality assigned by the sorter to

[1] This comparison is legitimate because the sorter always puts the wool from the shoulder areas into the highest qualities. It is interesting to note also that McMutrie found the shoulder areas the finest, if we leave out the "bellies" which are too short to go in best quality.

each fleece is plotted against the fineness of fibre as given by the T_1 average.

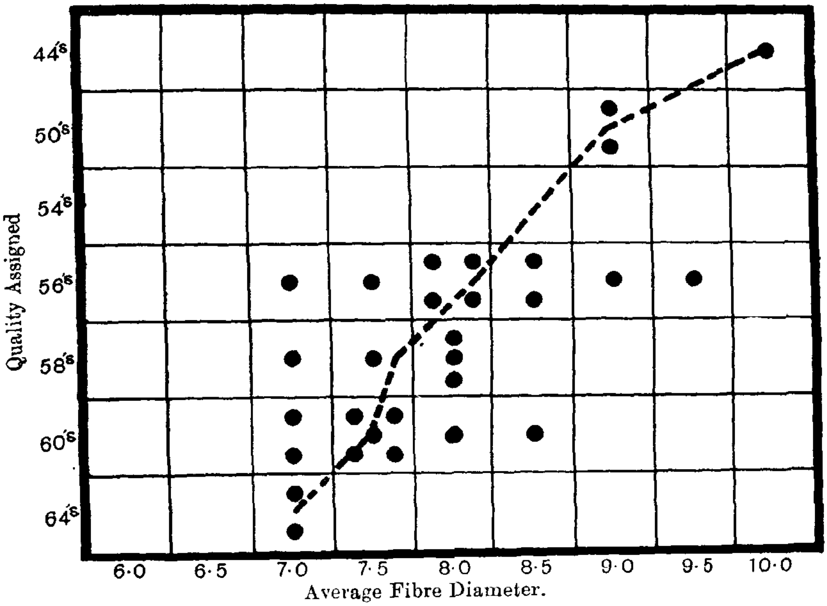


Fig. 5.

The dots inside the diagram indicate how many animals fall into each class. The broken line passes through the means of the rows.

From the above diagram it is obvious that in general with rise of quality comes decrease in the average T_1 diameter.

This result is confirmed by considering the corresponding measurements of the previous year, which are given below, the fineness average being given in $\frac{1}{1000}$ths of an inch.

60's	58's	56's	54's	50's
1·029	1·064	1·068	1·100	1·118.

However, in each case, although the averages show an increase with falling quality, it is found that averages for individual sheep of the same highest quality showed considerable fluctuations, the extreme individuals of any one quality-class often having a fineness of the same order as sheep placed by "sorting" in either a higher or lower quality class. F_1 ♀ 18 and F_2 ♂ 58 are marked examples of this overlapping tendency, as is seen in the table given below, in which each average is based on 60 measurements.

TABLE V.

	Quality assigned by sorter	T_1 Average[1]	T_3 Average[1]
F_1 ♀ 18	60's	8·72	6·05
F_2 ♂ 58	56's	7·36	5·97

These facts were of great interest, and consequently a further analysis of them was made in hopes of throwing light upon the modifying factors influencing the value of the T_1 average as a basis for commercial sorting.

An examination of the samples from F_1 ♀ 18 at once revealed a marked line of weakness in the fleece due to disease. The measurements of T_3 showed a low average. Consequently it may be concluded that genetically F_1 ♀ 18 is of a comparatively low quality, but that commercially it is classed as a comparatively high quality owing to pathological causes.

The case of F_2 ♂ 58 is more interesting as it appears to involve new considerations. It was found that the wool did not owe its low commercial quality to a high T_3 average. But further investigation showed that the wool was remarkable for its lustre, length, and the small number of crimps per inch, factors generally correlated with a high average diameter.

McMutrie has shown that a correlation does exist between the number of crimps per inch and the average diameter, but that there are exceptions. As this is a point of importance it was decided to collect McMutrie's figures into a form which should graphically display this relationship. This has been done in the two figures which follow. Fig. 6 is an ordinary two-fold correlation table, the numbers in the squares indicating the observed cases which belong to the different classes. The means of the averages—rows and columns—for this table were then calculated. They are shown in Fig. 7, the crosses (+) indicating the means of columns, the dots (•) the means of rows.

Considering the means of rows, it appears that these tend to lie on a curved line of regression which, in its upper part, is almost a vertical straight line. If the true line of regression be of this form, we may conclude that after the number of crimps per inch has reached about 23, the fineness of the fibre fails to change proportionately to the rise in the number of crimps although such a proportionate change occurs before this point. Confirmation is, however, not furnished by the other line of regression—the line about which the means of columns lie—and, in fact, the smallness of the number of observations renders a decisive

[1] These averages were confirmed by additional measurements.

conclusion unsafe. Nevertheless the nature of the lines of regression warrants further inquiry into the nature of the relationship.

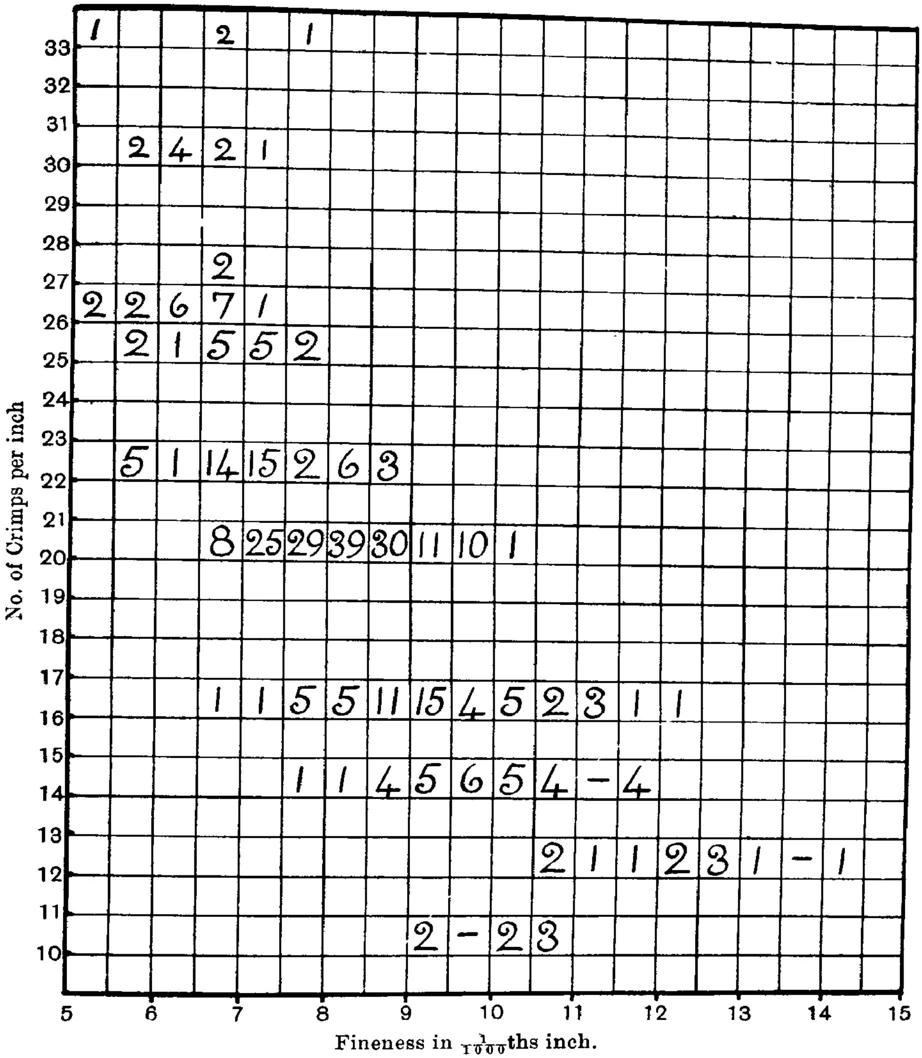


Fig. 6.

It seems certain that although there is in general a close connection between fineness of fibre and number of crimps per inch, cases occur where this relationship breaks down, a case in point being apparently the famous Mauchamp Merino. Where this breaking apart is marked, the relationship between fineness of fibre and commercial quality is likely

to become disarranged. F_2 ♂ 58 seems to be such a case, and it is of interest from a genetic point of view to note that the breaking apart of these two characters has occurred in an F_2 animal; but at present this fact must not be taken too seriously.

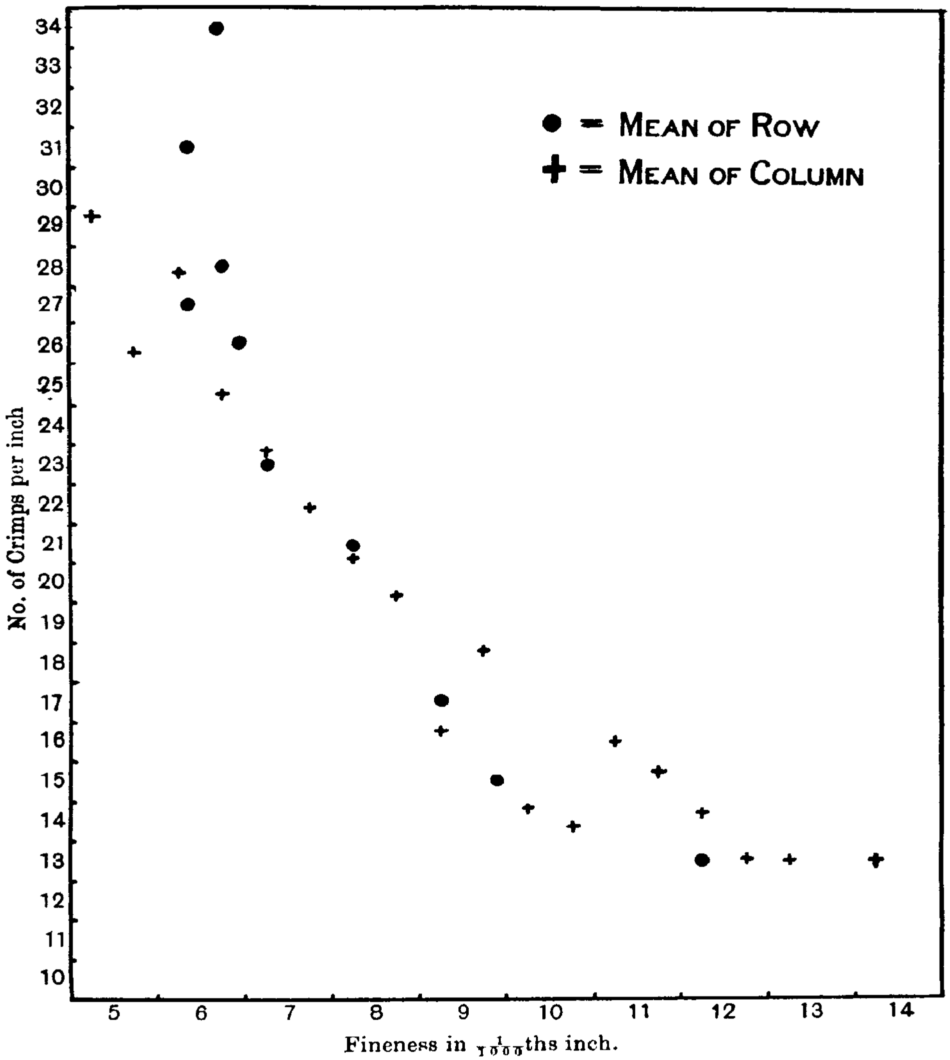


Fig. 7.

Up till now we have considered, following other observers, only the relationship between commercial quality of the sample and the average diameter of the fibres. The average diameter of the fibres in the sample

gives no indication of the variation in the diameter sizes in the sample. As has been stated above (p. 359), the sorter, in determining the quality of sample, is influenced by the presence of any obviously coarse fibres.

Now two samples might have the same average diameter, but one of them might have that average based upon a comparatively uniform collection of diameter sizes whilst the other might be based upon a wide range of diameter sizes, the coarse fibres being balanced by much finer fibres. The latter sample, owing to the obviously coarse fibres in it, would be classed as a lower commercial quality by the sorter than the former, although its average diameter is the same.

It occurred to us that the standard deviation of the fibre-diameter distribution in the sample should give a measure of this factor.

In Table III, as has been stated above, are given the qualities, the average diameter sizes of the fibres in those qualities, and the standard deviations of the distributions. An examination of the figures there given shows that in the case of T_1 there is a distinct parallelism between the quality assigned by the sorter to the sample, and the standard deviation of that sample, a fall in "quality" being accompanied in general not only by a rise in the average diameter size but also by a rise in the standard deviation. This latter rise cannot be explained as being wholly due to the rise in the average diameter as is shown by the fact that there is also a rise in the coefficient of variation $\left(\frac{\sigma}{M}\right)$.

In the case of the T_3 measurements the relationship between "quality" and the standard deviation holds in every class. Consequently it seems safe to assume that the standard deviation of a sample is of importance in modifying the conclusions drawn from this average as to the commercial quality of the sample.

Owing to the importance of this conclusion, and also owing to the possible interest from a genetic point of view, it was determined to investigate the question of influence of variability of sample on the commercial quality in more detail than by a mere examination of the standard deviations. In order to do this the percentage occurrence of diameters of every size was calculated for all the qualities.

The results are contained in Table VI.

In this table the "quality" slides are, in the case of every quality, the slides prepared for T_1 from the quality sample which the sorter selected for us. By "sheep of the quality" is implied all those sheep whose finest wool was of the quality concerned. The distribution of these sheep is, of course, given in Table V (above).

An examination of the table reveals several points of interest. It will be convenient to consider these points in the following order.

(i) *The qualities are seen to group themselves into four classes.*

Class I. The 60's and 58's. The fibre-distributions for these two qualities are very alike and show a comparatively small dispersion.

Class II. The 56's. The distribution for this class is of the same general nature as those of the 60's and 58's, but shows a distinctly greater dispersion.

Class III. The 54's and 50's. The distribution for this class appears to be bimodal, but this bimodality is not very pronounced, consequently we cannot be certain that it is really significant, although it is certainly suggestive. Apart from the question of the bimodality it is obvious that the range of variability is much increased.

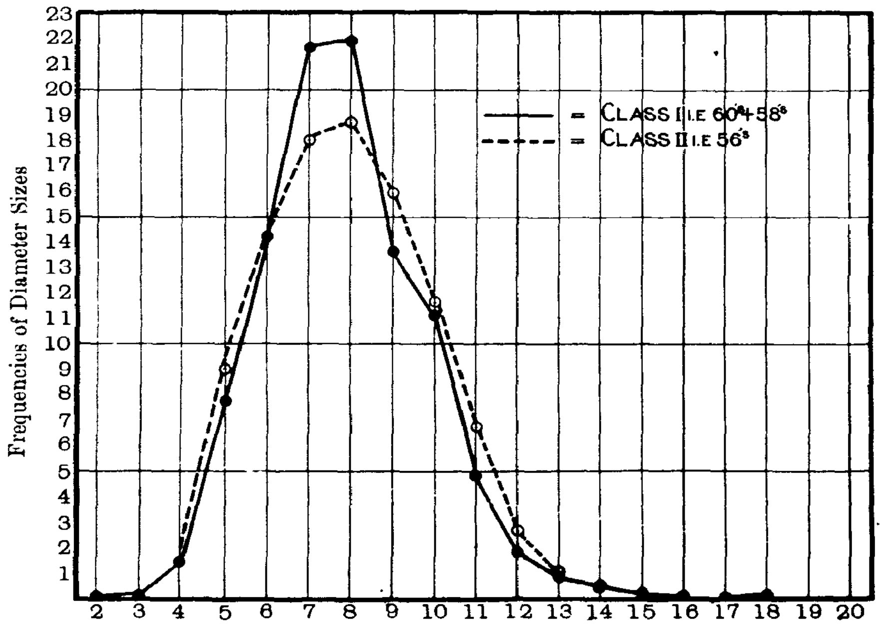


Fig. 8.

Class IV. There appear to be two or three modes here but the prominent lack of agreement between the quality slide and sheep distributions for the class destroys their significance. The 44's quality was, for the 1913 clip, one in which no great reliance could be placed for so few animals contributed to it. The fleece of F_1 ♂ 13 was consigned *en masse* to the 44's—a point of considerable genetic interest—and apart

Table VI.

Quality	Source of Measurements	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	No. of measurements made
		Diameters of Fibres																			
60's	Quality Slides	—	0·3	—	2·5	9·4	21·3	**31·3**	19·4	10·3	3·4	1·9	—	0·3	—	—	—	—	—	—	320
	Sheep of the Quality	0·1	0·3	1·3	7·7	14·3	**22·0**	21·6	13·2	11·1	5·0	1·9	0·9	0·4	0·1	0·1	—	0·04	—	—	2560
	Combine	*0·7*	*0·28*	*1·15*	*7·12*	*13·8*	*21·9*	***22·6***	*13·9*	*11·0*	*4·8*	*1·9*	*0·80*	*0·38*	*0·1*	*0·1*	—	*0·04*	—	—	*2880*
58's	Quality Slides	—	—	1·2	8·4	16·3	**24·1**	20·6	12·2	10·9	3·6	1·5	—	0·3	0·3	—	—	—	—	—	320
	Sheep of the Quality	0·1	0·2	1·9	8·8	14·1	20·2	**21·0**	13·0	11·3	5·0	1·8	1·1	0·7	0·4	—	—	—	—	—	1360
	Combine	*0·1*	*0·2*	*1·8*	*8·7*	*14·7*	***20·9***	***20·9***	*12·9*	*11·3*	*4·8*	*1·8*	*0·9*	*0·7*	*0·4*	—	—	*0·1*	—	—	*1680*
56's	Quality Slides	—	0·6	0·9	7·5	9·7	13·8	**23·4**	19·7	11·9	8·1	2·7	0·9	0·3	0·3	—	—	—	—	—	320
	Sheep of the Quality	0·1	0·3	1·6	9·4	15·3	**19·1**	17·4	14·9	11·6	6·4	2·5	0·9	0·3	0·3	—	—	—	—	—	1160
	Combine	*0·1*	*0·3*	*1·5*	*9·0*	*14·1*	*18·0*	***18·7***	*15·9*	*11·6*	*6·8*	*2·6*	*0·9*	*0·3*	*0·3*	—	—	—	—	—	*1480*
54's	Quality Slides	—	—	0·3	0·9	5·3	12·2	**18·8**	16·6	**18·4**	10·3	7·5	4·1	3·4	1·9	0·3	—	—	—	—	320
	Sheep of the Quality	No animal had 54's as its highest quality																			
	Combine	—	—	*0·3*	*0·9*	*5·3*	*12·2*	**18·8**	*16·6*	**18·4**	*10·3*	*7·5*	*4·1*	*3·4*	*1·9*	*0·3*	—	—	—	—	*320*
50's	Quality Slides	—	—	0·9	1·9	7·2	19·4	**19·7**	12·5	**17·2**	9·4	5·3	2·5	2·2	1·2	0·6	—	—	—	—	320
	Sheep of the Quality	—	0·2	0·2	1·1	6·3	**18·4**	13·8	14·1	**16·1**	11·7	8·3	6·0	2·8	0·9	—	—	—	—	—	640
	Combine	—	*0·1*	*0·4*	*1·4*	*6·9*	**18·8**	*15·6*	*13·5*	**16·5**	*10·9*	*7·3*	*4·8*	*2·6*	*1·0*	*0·2*	—	—	—	—	*960*
44's	Quality Slides	—	0·3	—	2·5	14·7	**20·6**	14·1	8·4	7·2	5·9	3·6	2·8	4·4	5·6	**6·6**	1·2	0·6	0·9	0·3	320
	Sheep of the Quality	—	0·3	0·6	2·2	3·9	8·1	8·8	**12·5**	10·3	13·8	**19·4**	8·8	3·9	**5·3**	1·2	0·2	0·2	—	—	320
	Combine	—	*0·3*	*0·3*	*2·3*	*9·4*	**14·4**	*11·4*	*10·5*	*8·8*	*9·8*	**11·4**	*5·8*	*4·2*	**5·5**	*3·9*	*0·8*	*0·5*	*0·5*	*0·2*	640

The modal frequencies are printed in heavy type.
The numbers in this table are the percentages of fibres of different sizes.

from this the class contained britch wool only. Hence it was of a very different type from the other classes and could hardly be expected to show the agreement between its distributions which they exhibit. The marked shift of its total distribution towards coarseness is the only point that we wish to emphasise at present.

In order to illustrate the nature of these four classes the curves of fibre-distribution for the classes are given in Figs. 8 and 9.

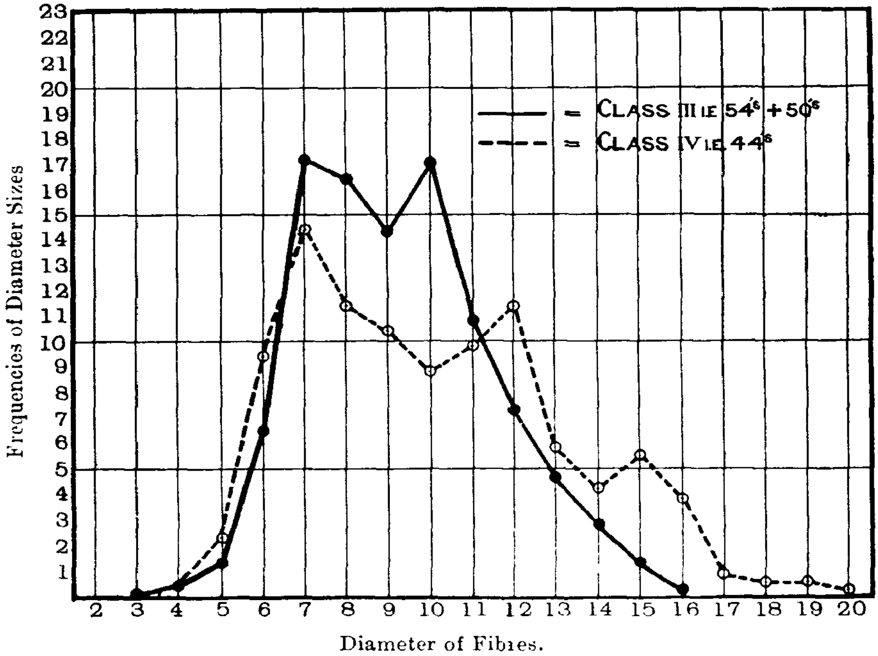


Fig. 9.

(ii) In every case the curves for the qualities have a modal point within the range given for the curves for the 60's and 58's. The lower qualities appear to have a second modal point falling outside this range. This fact is suggestive that all the samples considered have fundamentally one modal diameter size in common, the lower qualities owing their position to the occurrence of a second set of fibres having their range about another modal size outside the range of the 60's and 58's.

It should be stated at once that at present the facts are only suggestive, and also that the conclusion, even if correct, can only be applied to the present case, as the sheep from which the samples came were abnormal in that they were derived from a cross between two distinct

breeds. But the point is one of considerable importance, especially from a genetic point of view, and it is hoped to follow up these results by an examination of sheep of pure races as well as those of hybrid origin.

Summary and Conclusions.

(1) The method of taking four sub-samples and making in all 160 measurements of these sub-samples gives a satisfactory value for the average diameter of the sample.

(2) The average of the samples from each shoulder gives a good indication of the shoulder for each sheep.

(3) In comparing two sheep A and B we may take as almost certainly significant a difference between their two average shoulder diameters such that the ratio

$$\frac{\mathrm{M}_A - \mathrm{M}_B}{\epsilon_{AB}} > 3,$$

that is a difference of some 8 % of the average shoulder diameter of either of them for measurements taken as here indicated.

(4) A relationship exists between the fineness as measured by the average diameter and the commercial quality into which the wool is graded. But this relationship is not absolute and is not modified by various other factors.

(5) The average T_1 diameter (diameter at the tip) is the best guide from a genetic point of view as to the fineness of the wool concerned, owing to the marked pathological influences which may affect the T_3 (base) average.

(6) The distribution of the fibres of different sizes has a modifying effect upon the commercial quality which would be assigned from a consideration of the average size only.

It is suggested that the standard deviation of the distribution of the fibres should be used as a measure of this modifying effect.

In conclusion we desire to express our thanks to Professor Wood and Mr K. J. J. Mackenzie for placing at our disposal the animals dealt with in this paper.

Professor Barker has assisted us with much useful advice upon the commercial side of our work. In this respect, too, we are much indebted to Mr Dumville and Mr Hanson of Bradford.

To Mr G. Udny Yule we are much indebted for kindly criticising this paper.

LITERATURE.

1. NATHUSIUS (*a*) *Die Wollhaare des Schafes.*
(*b*) *Der Vorgang der Vererbung bei Hausthiere.*
2. BOHM. *Die Schafzucht.* (Berlin, 1874—83.)
3. KONIGSBOM. *Das Wollhaar der Schafe.*
4. BOWMAN. *The Structure of the Wool Fibre.*
5. MCMUTRIE. "Wool and Other Animal Fibres." (*Dep. Agric. U.S.A.*)

LAX AND DENSE-EARED WHEATS.

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THE present paper is written with no pretence at finality, and consequently some excuse is due for its publication at the present time. The author was induced to write it since the results so far obtained by him do not seem to agree, in all points, with the work previously done on the subject, and tend to show that there is much scope for future work both on this matter and also on a matter of much greater general importance, namely, on the influence of external conditions on a quantitative character.

Before proceeding further, it may be as well to explain the meaning of the terms "Lax" and "Dense."

Among the various wheats cultivated in different parts of the world, a character which is largely used to distinguish one variety from another is the shape of the ear. The most striking differences are found both in the length of the rachis of the ear (on which the spikelets are set) and also in the proximity of one spikelet to another.

Considering wheat as a whole, the number of internodes varies to a much smaller extent than does the total length of the ear, consequently, one may say, roughly, that if the rachis be short, the spikelets are pressed closely together, and if it be long, there is some space between one spikelet and the others above and below it.

Typical of the short, or dense ear, are all the *Compactum* wheats, while Polish wheat has the long, or lax ear; but one finds an almost unbroken series intermediate between these two extremes among the *Vulgare* wheats.

There have been two main methods of classifying wheats according to their degrees of density; the first is that used by the Research

Station at Svälof, which consists of grouping wheats into seven types according to their density as estimated by eye, Type 1 being the most compact, and Type 7 being the most lax. This practice was not found to be a great success owing to the impossibility of accurately defining the limits of the different types. The second method has had much more universal recognition, and is much superior to the one previously mentioned. It consists of finding the average space between the spikelets of the ear, in millimetres, and using this figure to denote the density of the ear. This is the method used by Nilsson-Ehle in his hybridisation experiments on this character.

According to this classification the *Compactum* wheats have a density of about 2·1 or 2·2 mm., while Polish has a density of about 6·6 mm.

The latter method was used in the case of the material under discussion,—whether satisfactorily or no will be discussed below.

The crosses which are here described were made in the summer of 1909 by Professor Biffen. Their original object was the continuation of his investigations on the inheritance of susceptibility and immunity to the attacks of the fungoid disease *Puccinia Glumarum* or Yellow Rust. The material, however, displayed so much diversity in the density of the ears of different plants that it seemed to be a very good opportunity for investigating the mode of inheritance of this character, and it was ultimately used solely for this purpose.

Several investigators have already worked on this problem, and their results are summarised below.

Professor W. Spillman seems to have been the first investigator, and his results are given very fully by C. E. Hurst[1]. Spillman's results are the more interesting in that the experiments were carried out before Mendel's paper on hybridisation was re-discovered. They were, in fact, started in 1899. In his experiments one parent was always a *Compactum*, while the other was a *Vulgare* wheat. A summary of his results for F_2 is given below. The percentages given are deduced by me from his data.

In these lax × dense crosses Spillman states that out of 108 F_1's, 97 were intermediate, 6 were lax and 5 were dense. With regard to the latter two groups it is not stated whether the seed-parents of the lax were lax, and of the dense were dense; but as no record is given of these two classes having been grown on, it seems probable that Spillman considered them as being due to selfing, instead of the crosses

[1] *Journal Royal Horticultural Society*, XXVII. 1902—3, p. 876.

having "taken." This seems the more probable in that out of the total 215 F_1's of all sorts mentioned in this paper, Spillman states that 66 were identical with the female parent, and were discarded as not being hybrids.

		% lax	% intermediate	% dense
2 plots	Emporium × Little Club	27·3	17·9	54·8
22 ,,	Jones Winter Fife × Little Club	26·9	47·4	25·7
3 ,,	White Track × Little Club	26·8	53·1	20·1
3 ,,	Valley × Little Club	33·8	49·5	16·7
6 ,,	Little Club × Emporium	26·0	31·0	43·0
7 ,,	Farquhar × Little Club	27·8	55·1	17·1
19 ,,	Little Club × Farquhar	27·5	48·8	23·7
11 ,,	Little Club × Valley	24·2	49·3	26·5
7 ,,	Turkey × Little Club	25·3	50·5	24·2
2 ,,	White Track × Red Chaff	30·6	49·5	19·9
4 ,,	McPherson × Red Chaff	24·0	39·7	36·3
7 ,,	Jones Winter Fife × Red Chaff	27·2	52·9	19·9
1 ,,	Farquhar × Red Chaff	30·7	52·5	16·8
3 ,,	Lehigh × Red Chaff	22·6	63·2	14·2
		380·7	660·4	358·9
	Average	27·2 %	47·2 %	25·6 %

The 97 intermediate F_1's were sown and selfed, and the above F_2's were the result. Two of the plots of the cross Little Club × Farquhar gave the unexpected result of yielding no dense, but 75 % intermediate : 25 % lax. These have been included in the above table but their behaviour is, after this lapse of time, inexplicable, as no details are given of their subsequent behaviour.

It will be seen from the table that there is considerable fluctuation in the individual percentages, but they distinctly suggest a simple 1 : 2 : 1 ratio.

Nilsson-Ehle noticed in 1900 that there was segregation in ear-shape on crossing, and his conclusions were published by Tschermak[1].

In consequence of Tschermak's report, which first drew attention to the occurrence of Mendelian segregation in various characters of the cereals, the value of Rimpau's work (1891) was much enhanced. The work of the latter gave distinct evidence that "splitting" occurred, but,

[1] "Ueber Züchtung neuer Getreiderassen mittlst künstlicher Kreuzung," *Zeitschr. für das Landw. Versuchswesen in Oestreich*, 1901, p. 1029.

owing to the small amount of material used, the ratios could not be deduced.

Nilsson-Ehle continued his work, and published his results in two papers written in the years 1909 and 1911[1]. A summary of his results is as follows. In crossing Schwedisches Binkelweizen (*Compactum*) with a lax-eared local wheat, he obtained a dense F_1 and splitting in F_2 into 3 *Compactum* (or *Compactum*-like) to 1 lax; and in all his crosses of *Compactum* × a lax wheat he got either a 3 : 1, or a 1 : 2 : 1 ratio, with *Compactum* more or less dominant, and he always found the parental types more or less faithfully reproduced in the F_2 generation.

When he crossed *Compactum* with a Squarehead type he got *Compactum* as dominant, but the Squarehead type hardly ever appeared in the F_2, the lax descendants being almost all more lax than Squarehead. To explain this he points out that many workers have found that when Squarehead types are crossed with lax-eared wheats, lax is dominant: thus, the lax types possess a factor for length which is lacking in the Squarehead types. If this factor be represented by L, the lax-eared wheats are L, and the Squarehead are l. Now when lax is crossed with *Compactum*, the latter, as we have seen, is dominant, and therefore contains a factor C which is absent from the lax wheat. If we suppose that *Compactum* is of the constitution CL, and that the lax-eared wheat is of the constitution cL, in F_2 we only get lax and dense of the two parental types. If the constitution of Squarehead is cl (since it contains neither the *Compactum* factor nor the one for length) we obtain, on crossing it with *Compactum*, an F_2 consisting of the following proportions:

9 CL (*Compactum*) : 3 Cl (*Compactum*) : 3cL (lax) : 1 cl (Squarehead).

This means that there is only one plant of the Squarehead type in every 16 plants, but in Nilsson-Ehle's results it occurred even less often than this. To explain this he suggests that there are two lengthening factors in *Compactum*, both masked by the factor C, each being inherited independently of the other, and neither being present in Squarehead types of wheat. This would mean that the Squarehead type would only recur once in every 64 plants in F_2 of a cross between *Compactum* and Squarehead, and all the rest of the lax would be more lax than Squarehead.

Nilsson-Ehle's conclusions were as follows:

1. The heritable gradations of a length character are the result

[1] "Kreuzungsuntersuchungen an Hafer und Weizen," *Lunds Universitets Årsskrift*. N. F. Afd. 2, Bd. v, Nr. 2, p. 104 *et seq.*; *Ibid.* Bd. vii, Nr. 6, p. 26 *et seq.*

of the accumulated action of several factors which segregate in a Mendelian manner.

2. These factors are of two kinds; some are positive lengthening factors, some are, on the other hand, factors which inhibit length.

3. Through the operation of an epistatic factor a discontinuous segregation can be turned into a continuous one, exhibiting an unbroken gradation of forms joining the two extremes. Through the grouping together of different segregating factors a constant heritable "variation" can make its appearance from dissimilar crosses.

4. The occurrence of gradations which lie outside the limits of either parent, where the two parents are dissimilar in quantitative characters, is explained by the re-grouping of factors which segregate in the ordinary Mendelian manner.

Wilson[1] crossed Red King (lax) with Rood Koren (*Compactum*) and obtained a *Compactum* F_1; F_2 gave a distinct ratio of 3 dense : 1 lax. He noted, however, that there were different degrees of density among the dense group, the less dense being in the majority.

Strampelli[2] obtained, from crossing Herisson (*Compactum*) with Rieti (lax) an F_1 consisting mostly of Squarehead types which approached more nearly to the *Compactum* parent; in the F_2 the usual splitting into *Compactum*, intermediate, and lax, took place.

v. Rümker obtained, from a cross between *Compactum* and lax "Eppweizen" an F_1 of intermediate Squarehead type, and in F_2, quite a distinct ratio of 1 *Compactum* : 2 Squarehead : 1 lax. The Squarehead types, when sown, gave the same splitting in their descendants. The other crosses which v. Rümker made with *Compactum*, showed signs, from their behaviour in respect to other characters such as beards, that the parents used were not constant, but heterozygotes, and the crosses gave, therefore, no reliable information with regard to the inheritance of laxness.

Tschermak states that in all his *Compactum* crosses, *Compactum* was always dominant.

Biffen's earlier experiments with lax × dense wheats are given in his paper, "Mendel's Laws of Inheritance and Wheat Breeding[3]." He describes three crosses which he had made. The first was between Squareheads Master and Red King. The internode length of the

[1] *Journ. Agric. Sci.*, II. 1, 1907, p. 82.

[2] "Alla ricerca e creazione di nuove varietà di frumenti a mezzo del' ibridazione," *R. staz. sperim. di granicoltura in Rieti*, Roma, 1907.

[3] *Journ. Agric. Sci.*, I. 1, p. 30.

former was 3·2, and that of the latter was 4·6 mm. The F_1 was more lax than the lax parent, having an internode length of 4·8. The F_2 were divided into plants having an internode length of under 4·6, and those having an internode length of 4·6 or over. With this classification a ratio of 22 dense : 78 lax (or 1 : 3·5) was obtained. The limits of the lax group were beyond those of the lax parent, but no plants were found which were more dense than the dense parent.

The second cross described was between Rivet (3·6) and Polish (6·6). The F_1 had an internode length of 5·8, and the F_2 consisted of a series with internodes from 3·1 to 6·8 mm. The ratio was calculated as 130 dense : 362 intermediate : 179 lax, or 19·4 %$_0$: 53·9 %$_0$: 26·7 %$_0$. In the third cross the two parents were Devon (moderately lax) and Hedgehog (*Compactum*) but only the F_1 is mentioned. This is described as intermediate, but, from an unpublished photograph, it appears to approach much more closely to the *Compactum* parent.

As will be seen, it is very hard to draw any general conclusions concerning the genetics of these characters from the work previously done. We find that Spillman, Strampelli, and v. Rümker all get an F_1 of intermediate character, and an F_2 of 1 dense : 2 intermediate : 1 lax; while Wilson and Tschermak find dense to be distinctly dominant in F_1 and the former got a ratio of 3 dense : 1 lax in F_2. Nilsson-Ehle and Biffen obtained different results from different crosses, the former sometimes found the F_1 intermediate, when the F_2 always gave a ratio of 1 dense : 2 intermediate : 1 lax, and sometimes found the F_1 similar to the dense parent, when the F_2 showed a ratio of 3 dense : 1 lax. Biffen found lax dominant to medium dense, and the F_2 giving 3 lax : 1 medium dense, in one case; and in another case he got an F_1 intermediate between the two parents, and the F_2 appeared to show a ratio of 1 : 2 : 1. Where he crossed a *Compactum* with a medium lax wheat he found slight dominance of the dense character.

Nilsson-Ehle is the only investigator to attempt to enunciate a theory which could reconcile these different results, but, as will be seen, the present case, at least, does not strengthen the evidence on which his theory is based.

It may be pointed out in passing, that the two last-mentioned investigators are the only ones to use measurement to analyze their results.

In the present investigation, two crosses were utilised, both of them having a wheat known as American Club as one parent, the other parent in each case being a wheat with ears of moderate laxity. These were Square Ghurka and Square White.

American Club is a typical *Compactum* wheat, while Square White and Square Ghurka are both *Vulgare* wheats of hybrid origin, but which have bred true to type for a series of years.

It must be emphasised that the latter two wheats would be classed according to Nilsson-Ehle as lax, and not as Squareheads type, as those which he describes as Squarehead have an internode length of 3·0—3·7 whereas Square Ghurka has an internode length of 3·9—5·1, and Square White, though no measurements can be given, is practically identical in this respect.

At the beginning of the investigation of the F_2's from these crosses, it was thought that the material could be quite satisfactorily investigated by eye alone, and all the material provided by the cross Square White × American Club was exhausted before the inadequacy of this method was established. It was found that the plants could not be divided into definite groups, as was thought at first, but that they formed a continuous series varying from very lax to very dense. As a result of this, it was decided to submit the material from the other cross—Square Ghurka × American Club—to accurate measurement, by finding the internode length, as mentioned above.

The internode length of American Club was found to be from 1·9—2·5; while that of Square Ghurka was 3·9—5·1 mm.

Unfortunately it was not until the F_2 stage that it was decided to use this material for the investigation of this character, so that no accurate measurements are available in regard to the internode length of the F_1, but from a photograph, and from information supplied by Professor Biffen, it is safe to say that it was almost intermediate but that the tendency was towards the dense parent.

F_2 showed, even on casual inspection, that there were a large number of plants the ears of which were either more lax or more dense than were the ears of either parent. (It was this fact which first caused the decision to use the material for the investigation of this problem instead of that of the inheritance of disease resistance.)

As will be seen from Table I, the internode lengths varied between 1·5 and 5·7, but the curve (see Fig. p. 379) though continuous gives distinct evidence that segregation is taking place, the break between the lax series and the dense one seeming to lie somewhere about 3·2. It was decided to grow offspring from each plant of the F_2, to see, if possible, which of them were heterozygous and which homozygous. It will be seen below, that, judging by offspring, the break between homozygous and heterozygous ought to come at about 3·7.

TABLE I. *Showing the Distribution of Offspring in F_2, F_3 and F_4 from the Cross Square Ghurka × American Club, in reference to their Mean Internode Lengths. The internode lengths measured on the ear of the main tiller only.*

Mean internode length in mm.	F_2	F_3	F_4
1·4—1·5	2	3	10
1·6—1·7	8	17	46
1·8—1·9	19	98	82
2·0—2·1	13	161	81
2·2—2·3	15	243	92
2·4—2·5	17	311	80
2·6—2·7	14	257	63
2·8—2·9	4	226	34
3·0—3·1	1	153	17
3·2—3·3	—	115	20
3·4—3·5	1	79	19
3·6—3·7	1	41	27
3·8—3·9	2	66	45
4·0—4·1	4	46	50
4·2—4·3	5	44	54
4·4—4·5	5	68	55
4·6—4·7	4	97	42
4·8—4·9	1	138	46
5·0—5·1	8	147	22
5·2—5·3	2	146	25
5·4—5·5	5	125	17
5·6—5·7	1	111	6
5·8—5·9	—	89	3
6·0—6·1	—	60	3
6·2—6·3	—	46	1
6·4—6·5	—	19	1
6·6—6·7	—	23	—
6·8—6·9	—	9	—
7·0—7·1	—	3	—
7·2—7·3	—	7	—
7·4—7·5	—	—	—
7·6—7·7	—	1	—
7·8—7·9	—	—	—
8·0—8·1	—	—	—
8·2—8·3	—	—	—
8·4—8·5	—	—	—
8·6—8·7	—	1	—
Total	132	2950	941

Taking the point of division at 3·7 the ratio of dense to lax is 95 : 37 or 2·6 : 1. Taking into account the evidence of other investigators and the smallness of the numbers, these figures seem to be near enough to 3 : 1 (99 : 33) for one to assume as certain that this indicates the mode of inheritance for the two main divisions, but the occurrence of the very lax and very dense remains unexplained.

In F_3 nearly all of the lax-eared plants bred true to laxness, and a certain proportion of the dense-eared plants bred true to denseness, but it was very difficult to estimate the proportions of the latter, and

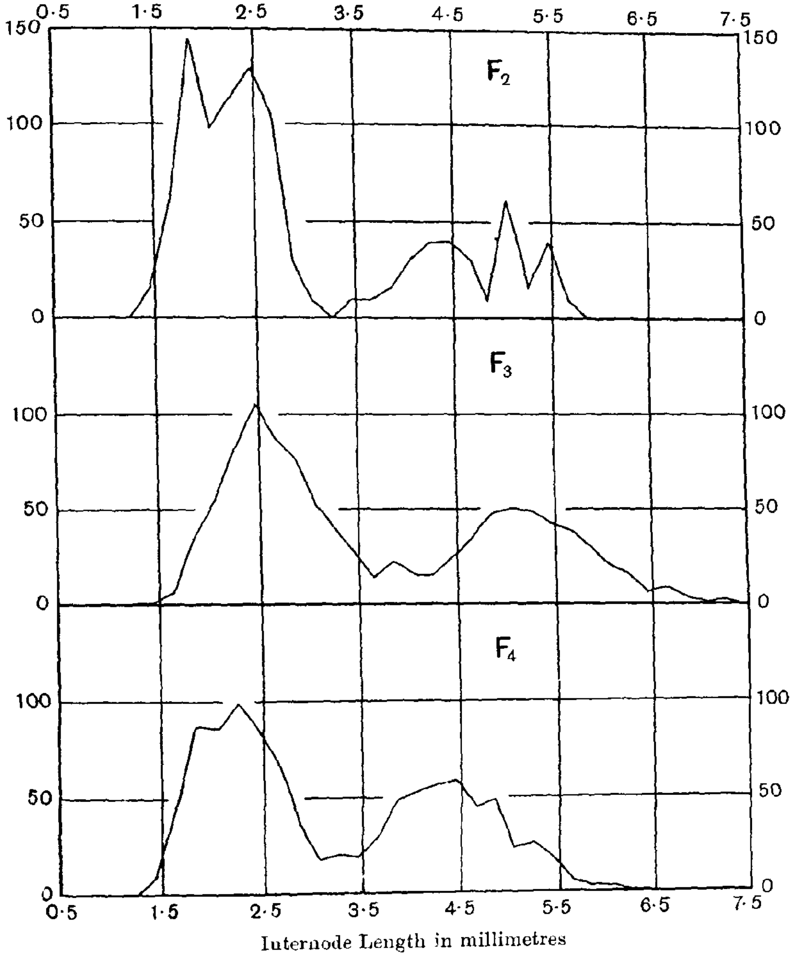

Figure showing the Distribution per Thousand of Offspring, in F_2, F_3, F_4 from the Cross Square Ghurka × American Club, in respect to Mean Internode Length. (Data of Table I, p. 378.)

practically impossible to see which plants bred true to their particular degree of denseness or laxness. A fact which is particularly noticeable is that, although a large proportion of the very dense eared plants threw no lax, there were several which were heterozygous. It is also

remarkable that the range of density widens still more in F_3 than it did in F_2; and that this broadening is particularly noticeable on the lax side.

Although the F_2 of the Square White × American Club cross were only sorted by eye, the general result of the investigation appeared to agree with that of the one in which measurement was used, and a sample of F_3 derived from this cross gave a curve almost identical with that of the Square Ghurka × American Club cross. These F_3 plants were also descendants of each of the F_2 plants of that series.

A noticeable feature of both the F_3 series is that the curves which they form have both shifted towards the high, or lax side.

From the Square Ghurka × American Club cross 36 F_3 plants were grown on, being chiefly selected either because they lay between the two peaks of the curve, or because they lay at the extremes of the curve. The internode length of their descendants is shown in Table III. The curve of their descendants is also shown, not that it gives one any direct idea of what the curve would have been if all the plants had been grown on, but because it indicates at about what point the curves intersect, and may be compared with those plotted for the two preceding years. The two peaks are here seen to have reverted almost exactly to the positions which they held in 1911. And this seems to bear out the theory that some such factor as the wetness of the summer of 1912 was responsible for the shift shown in the curves for that year.

From the form of the F_2 curve itself it would seem that there were about 71 °/₀ dense : 29 °/₀ lax. Judging from the progeny of these plants it would seem that the F_2 consisted of about 72 °/₀ dense. But the points of division chosen are quite arbitrary, as there is not any distinct break in the F_2 curve, and, as will be seen from the F_3 table, it is impossible to say with complete confidence which plants are giving dense offspring, are splitting, or are breeding true to laxness.

In this F_3, if the 3 dense : 1 lax ratio is a correct assumption, there ought to be 62 °/₀ dense : 38 °/₀ lax. Judging from the curve, the results fairly bear out the theory.

A fact which is noticeable in the tables showing the descendants of the F_2 and F_3 plants is that in the non-splitting dense and lax, specially in the former, there seems to be a distinct correlation between the degree of density of the parent, and the average degree of density of the offspring. This is particularly noticeable in the dense descendants of the F_3 parents. There can only be two possible theories to explain this phenomenon. In the first place it might be due to nutrition, for,

TABLE III. *Showing the number of offspring from certain selected Plants in F_3 with a given Mean Internode Length; the internode lengths measured on the ear of the main tiller only. F_3 plants ranked in order of internode lengths.*

Plant No.	F_3 Internode length in mm.	F_4 Internode lengths in mm.																									
		1·3—1·5	1·5—1·7	1·7—1·9	1·9—2·1	2·1—2·3	2·3—2·5	2·5—2·7	2·7—2·9	2·9—3·1	3·1—3·3	3·3—3·5	3·5—3·7	3·7—3·9	3·9—4·1	4·1—4·3	4·3—4·5	4·5—4·7	4·7—4·9	4·9—5·1	5·1—5·3	5·3—5·5	5·5—5·7	5·7—5·9	5·9—6·1	6·1—6·3	6·3—6·5
16	1·6	5	13	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	1·8	—	1	8	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22	1·9	1	5	12	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22	1·9	3	7	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18	1·9	—	6	9	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	1·9	1	5	4	3	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	2·5	—	—	2	6	7	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	2·5	—	—	3	13	13	6	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	2·5	—	—	2	5	11	7	7	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	2·5	—	—	3	3	4	4	—	—	—	—	—	2	2	2	—	—	—	1	1	—	—	—	—	—	—	—
29	2·5	—	3	13	4	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	2·5	—	2	—	1	4	5	2	1	—	—	—	—	1	—	1	1	—	—	1	—	—	—	—	—	—	—
27	2·5	—	—	—	—	1	5	13	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	2·7	—	2	6	8	6	4	1	—	—	—	—	—	1	—	4	2	1	—	—	—	—	—	—	—	—	—
27	3·3	—	—	—	—	—	—	1	4	—	1	1	5	2	—	—	1	—	—	3	1	2	2	—	—	—	—
29	3·3	—	—	6	1	9	11	2	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29	3·5	—	—	—	13	6	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
33	3·5	—	—	1	3	4	7	7	3	5	2	2	—	—	1	3	2	1	2	—	1	1	—	—	—	—	—
29	3·6	—	—	1	8	8	11	4	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	3·7	—	—	—	—	3	—	5	3	—	—	—	—	—	1	2	—	—	—	—	—	1	—	—	—	—	—
18	3·8	—	—	—	3	7	5	7	1	1	1	—	2	1	1	—	1	1	—	—	1	—	—	—	—	—	—
33	3·8	—	2	4	3	5	4	7	3	—	1	—	1	2	2	2	2	1	1	—	—	—	—	—	—	—	—
27	3·9	—	—	—	1	1	2	—	2	2	7	3	—	—	—	—	—	1	—	1	—	—	—	1	1	—	—
33	4·1	—	—	—	—	1	2	5	3	5	2	1	1	1	—	—	2	1	6	3	2	1	—	—	—	—	—
30	4·3	—	—	—	—	—	—	—	—	1	4	1	4	9	6	1	1	2	—	—	—	—	—	—	—	—	—
34	4·4	—	—	—	—	—	—	—	—	—	2	5	—	5	3	1	3	1	—	—	—	—	—	—	—	—	—
34	4·5	—	—	—	—	—	—	—	—	—	—	3	6	2	3	4	3	2	1	—	—	1	—	—	—	—	—
36	4·6	—	—	—	—	—	—	—	—	—	—	—	—	—	5	4	3	2	2	—	—	1	—	—	—	—	—
85	5·8	—	—	—	—	—	—	—	—	—	—	2	1	3	3	5	9	3	3	—	1	—	—	—	—	—	—
34	6·2	—	—	—	—	—	—	—	—	1	—	—	—	1	2	3	7	7	6	4	5	—	1	—	—	—	—
24	6·2	—	—	—	—	—	—	—	—	—	—	—	—	1	1	3	3	2	4	2	1	2	—	—	—	—	—
30	6·3	—	—	—	—	—	—	—	—	—	—	1	2	4	11	4	5	1	4	1	1	1	—	—	—	—	—
24	6·7	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	3	4	2	4	4	—	1	1	—	—
20	6·7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	4	5	1	4	2	3	1	1	—	1
34	7·1	—	—	—	—	—	—	—	—	—	—	—	1	7	7	11	6	7	6	2	1	1	—	—	—	—	—
97	8·6	—	—	—	—	—	—	—	—	—	—	—	1	3	2	4	2	2	1	1	3	1	—	—	—	1	—

owing probably to the cramped space in which the grain of the very compact plants develops, it is always appreciably lighter than that from ordinary ears, but, for this to have any effect on the density of the ears of the offspring, two assumptions have to be made, first, that weak plants develop from small grain, and secondly, that the ears of weak plants are appreciably more dense than those of normal plants. Against the first assumption Johannsen and many others have collected a large mass of evidence, and the second assumption, on the face of it, does not seem likely; at any rate it is hardly possible to imagine that such an appreciable difference as would be required to explain the correlation here observed would have been overlooked until now. It seems safe, therefore, to conclude that the alternative theory is the true one, namely, that underlying the main 3 : 1 ratio, there are other factors which have a modifying effect on the laxness or denseness of the ear. This is, probably, also the explanation of the appearance of plants which exceed the limits of either parent.

In comparing these results with those of Nilsson-Ehle, it is important to emphasize the fact that this cross is between a dense and a lax wheat; the latter parent being quite distinctly more lax than any Squarehead type. Although, as will be seen below, the average internode length is a figure which is practically useless as a standard for comparing the finer degrees of density, it is noticeable that while Nilsson-Ehle's Squarehead type has an average internode length of 3·4 mm., Square Ghurka was 4·4 mm., in fact Square Ghurka was 23 °/₀ more lax; this, though no proof, helps to confirm the conclusion already drawn from ocular comparison of Square Ghurka with several Squarehead types grown at Cambridge in the same year, under similar conditions. For example, Squareheads Master had a mean internode length of 3·9 mm., so that Square Ghurka was 11 °/₀ more lax. Now, according to Nilsson-Ehle's scheme, Dense × Lax should only reproduce the parental types and nothing else, whereas in the cross in question this is not the case. To satisfy his scheme it is essential to conclude that at least one more lengthening factor must be contributed by the Club parent, which is a possibility which he himself has suggested. His interpretation, however, does not, and, as far as one can see, cannot allow for the appearance of plants having more dense descendants than the dense parent, and there is every indication, in the case of the present cross, that such plants do arise. For example, if one follows the behaviour of plant No. 16, even though it eventually proved itself to be a heterozygote, it hardly seems probable that its density is of the

same quality as is that of Club. The F_2 plant had an internode length of 1·5 mm. Its descendants had a mean internode length of 2·1 mm. When four of these were grown on, one of them proved to be a heterozygote, but the other three had a mean internode length of 1·76 mm.; unfortunately the internode length of Club was recorded in only one of these years, 1912; in that year Club had an average internode length of 2·2, while the mean of No. 16 was 2·1 mm.; in fact No. 16 was 5 °/。 the denser of the two, when grown under identical conditions. It can hardly be doubted that if individual descendants of No. 16 were grown on, some of them would be found to breed true to a greater density than that of Club.

The author does not contend that the facts here enumerated disprove the possibility of Nilsson-Ehle's theory being correct, but he wishes to point out that, although just under 4000 plants were measured, the material proved to be insufficient to enable one to state whether it gave evidence that Nilsson-Ehle's theory, based on the examination of 1150 plants, be correct or false. One can merely consider it as not proven; nor will it be proven until a great deal more work has been done on the subject.

The main difficulty in obtaining satisfactory data for a decision as to what are the true factors involved in this and similar crosses is the great effect which external conditions have upon the density of the ears of wheat. As was mentioned above, the F_3 curve of the Square Ghurka × Club cross lies considerably further out on the lax side than do either the F_2 or F_4 curves. This shift in the curves coincides with a very wet autumn and spring, and, as far as can be seen, can only be ascribed to that cause. The rainfall registered for the period in which the crops of the two years were making their early growth is given below:

	1911—1912	1912—1913
November	2·64 inches	1·83 inches
December	3·52 ,,	2·05 ,,
January	3·24 ,,	2·22 ,,
February	1·04 ,,	·76 ,,
March	2·24 ,,	1·63 ,,
Total	12·68 ,,	8·49 ,,

These records were taken at the Cambridge University Botanical Gardens, about two miles from the University Farm. No records taken nearer the spot are available.

To gain further knowledge upon the effect of weather conditions it seemed important to investigate whether this effect were due to the

lengthening of the rachis, or to a decrease in the number of internodes, or to a combination of the two. It is unfortunate that it was impossible to compare the figures obtained in the wet year (1912) with those of 1911, for in 1911, only the record of the internode lengths was preserved, and the figures from which these internode lengths were deduced were not recorded; however, 1913 was a comparatively dry year, and the curve had shifted back to almost the same position in which it was in 1911, so the figures obtained in that year are here used for comparison. The mean of the group is, in each case, the figure given. The following table shows the results obtained. All families which contained no lax individual were classed as dense, and all families which contained no dense were classed as lax. Those families which contained both lax and dense were not utilised. As dense is dominant, the dense group of each year contains without doubt some heterozygotes, but as they are not distinguishable either by eye or measurement from the homozygotes, this fact can make no material difference to the conclusions to be drawn from the table.

	1912		1913	
	No. of Nodes	Rachis length	No. of Nodes	Rachis length
Lax	15·8	82·9	21·1	95·0
Dense	15·6	36·6	21·7	44·4
Squareheads Master	21·2	82·7	23·4	81·1

From the above, it will be seen that both the rachis length and the number of nodes have been diminished during the wet season, and that the increase in the average internode length is due to the external conditions having a bigger checking effect on the number of nodes than on the length of the rachis. The alteration is given below in °/ₒ.

Difference between 1912 *harvest and* 1913 *harvest.*

	No. of nodes	Rachis length
Lax	+33 °/ₒ	+15 °/ₒ
Dense	+39 ,,	+21 ,,
Squareheads Master	+10 ,,	– 2 ,,

The facts shown above are of great interest, in that the larger variation is a meristic variation, and that external conditions would seem to cause an alteration of nearly 40 °/ₒ in the number of parts. The extent of this variability is still more amazing in that, early in the spring, dissection of a young wheat plant shows the nodes already fully formed; thus, the conditions which control this variation can only

be operative during the early life of the plant. A practical interest is added when one considers that, other things being equal, the number of nodes controls the number of flowers on an ear, and these control the quantity of grain formed. From every point of view it would seem expedient that further investigations should be made on this subject.

The behaviour of a sample of Squareheads Master during the same two seasons has been added for comparison; its behaviour, as can be seen, is somewhat dissimilar to that of the hybrids in question, for, where their number of internodes shows a large increase in the year 1913, those of Squareheads Master have increased in number only by about 10 %, and, at the same time, the total rachis length has decreased by 2 %. Both of these facts may be due to sowing at a different time, or to some other cause not yet discovered, but the figures are also important in that they show that increase in the number of internodes does not necessarily mean that the length of the rachis is also increased, in fact that one cannot regard the two variations as both resulting from one and the same cause. The discrepancy is possibly caused by the fact that only the early weather conditions affect the number of internodes, while it seems likely that the weather conditions prevailing during the whole growing period of the plant would, together, control the length of the rachis. Thus, weather experienced after the earlier period of growth might nullify the effect caused by the weather of that time, and then the variation of the two characters would become practically independent.

This has an important bearing on the question of the practical value of the present methods of classifying wheats according to the density of their ears. As has already been shown, the method used at Svälof is inefficient for finer work. and the only alternative method is that used in the present investigation. But the average internode length—the standard here used—is the relation between the two above characters, the number of internodes and the total rachis length. Now it is obvious that these two vary largely from season to season, and if, as seems probable, they can vary independently of one another, and indeed in opposite directions, it might be quite possible to get a variability of well over 50 % in a variety from one year to another, and that even when grown on the same soil with identical manurial and cultural conditions. Such a character therefore is one the genetics of which are very difficult to deal with. Either of the characters treated alone might be less labile (affected by environmental conditions) than the ratio, and, of the two, rachis length seems to be less affected

than the number of nodes. However, neither of these characters, taken by itself, is an index of density, and neither can be used as such.

In a previous paper[1] it was shown that, in a large sample of Squareheads Master, there was a high correlation between the average internode length and the total rachis length, and it was suggested that the relation between these two characters might possibly be useful as a standard of density of the ear. In the light of the facts here set out, however, there is every reason to believe that the relation between these two characters might alter with changing external conditions.

The results here given, as respects the influence of external conditions, lose much of their importance from the fact that neither of the parent wheats were grown on as a standard of comparison, and, to a less extent, from the fact the material used, in the case of the dense wheat at least, was not homogeneous. The composition of the dense group, however, would not vary largely from year to year, and it may be noted that practically the same change was found in both the lax and the dense groups, which suggests very strongly that the difference was primarily, if not entirely, due to external influence.

This part of the investigation at least shows the necessity for further research which may prove exactly what effect weather has on these two characters.

In conclusion, it is hoped that this paper will have sufficiently clearly pointed out the fact that the problem of the inheritance of the characters of laxness and denseness in the ears of wheat is a much more complicated one than was previously imagined, and that the only possible way to solve it will be to start on the simplest cases, and, when they are solved, to proceed to the more complex. In consideration of the fact that there are probably minor factors underlying the main lax and dense factors, it will be essential to work with pure lines. The parents will have to be grown on from year to year for comparison. It is obvious that it will be necessary to deal with a far larger number of progeny than were used by Nilsson-Ehle or any of the previous investigators.

It is, therefore, proposed to continue the experiment by making extensive crosses between pure lines of lax and dense, lax and intermediate, and intermediate and dense wheats, and to grow on all the parental lines for comparison. It is also proposed to note the variability of the number of nodes, and of the total rachis length in pure lines from season to season, to attempt to throw more light on the effect of weather conditions on these characters.

[1] *Journ. Agric. Sci.*, IV. 2, p. 179.

VOLUME VI DECEMBER, 1914 PART IV

TOWN SMOKE AND PLANT GROWTH

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IN a previous communication[1] we have summarised the results obtained by us in a study of the nature and extent of atmospheric pollution in different parts of the city of Leeds and in further studies of some of the effects of smoke upon plant growth.

Our results indicated that the growth of plants in the city is likely to be markedly affected by the following three factors amongst others attributable to smoke:

1. The reduction of the available solar energy, amounting in the worst case to as much as 40 per cent.

2. The reduction of the assimilatory powers of the leaves of plants, partly owing to the thick black superficial deposit causing further reduction of available solar energy, and partly to actual choking of stomata by sticky particles of soot.

3. The presence of free acid in the air and brought down by rain, causing direct damage to the plant through corrosion of leaf-tissue and indirect damage by way of the soil, where the latter is poor in calcium carbonate.

With this evidence before us we decided to attempt to measure directly the inhibiting effects of atmospheric pollution upon plant-growth in different parts of the city, in the hope that it might prove possible to establish some degree of correlation of the actual growth of plants under uniform soil conditions, with the previously-ascertained degrees of atmospheric pollution in these different parts.

In the spring of 1911 six experimental stations were selected, five of which were situated within the boundaries of the city of Leeds, and the remaining one at Garforth, some six miles due east of Leeds.

[1] This *Journal*, IV. 25.

Of the stations in Leeds one (Hunslet) was situated in the very heart of the most highly-polluted industrial area, about one mile south of the centre of the city, whilst the other four stations were roughly in line to the north of this at successive distances apart of about a mile. The relative situations of the various stations are summarised in Table I, which includes also data from our earlier paper indicative of the degree of pollution of the atmosphere in each district.

TABLE I.

Station			Annual Precipitation, lb. per acre			
No.	Locality	Position	Total suspended Matter	Total Sulphur expressed as SO_3	Free Acid expressed as H_2SO_4	Chlorine
1.	Hunslet	Industrial Area	1565	215	90	198
2.	Park Square	Heart of City	849	197	45	75
3.	University	1 mile north	399	134	26	51
4.	Headingley Hill[1]	2 miles north	273	103	19	43
5.	Weetwood Lane	3 miles north	147	73	11	34
6.	Garforth	6 miles east	—	91	28	22

[1] This station lies roughly half-way between stations 3 and 5, and the data given are the means of the results obtained at these stations.

For the purposes of the experiment a quantity of soil, poor in calcium carbonate, was taken from one of the fields of the Experimental Farm at Garforth, intimately mixed and then used for filling 18 large wooden buckets each holding about 100 lb. of soil. These were then distributed, three to each centre, the "centre" in each case being a large garden or other supervised open space. The boxes were there sunk in the soil, leaving a projecting rim of 1—2 inches. On April 4th, 1911 all the boxes were sown with radishes (var. French Breakfast) and Cos Lettuce in the following manner. After making a fine seed-bed 75 radish seeds were uniformly distributed over the soil-surface in each bucket, covered with a light layer of sifted soil, on which 20 seeds of lettuce were then sown and these in turn covered with fine soil so that the radish seeds were covered by a half-inch layer of soil. When the sowing was completed the buckets were well watered to ensure a favourable start, and it was intended to depend solely upon the rainfall for further supplies of moisture. The dryness of the season was, however, such that it was thought necessary to water the plants on three other occasions. In each of these cases the buckets were all watered on the same day, the

same measured amount of water being sprinkled on each. Periodical visits were paid to the stations, but the plants were not interfered with in any way.

TABLE II. *First Crop, Radishes.* (Cp. Fig. 1.)

Station	Green weight		Dry matter		
	Total weight	Average weight per plant	Total weight	Percent. of green weight	Percent. of SO_3 in dry matter
	gms.	gms.	gms.	%	%
1. Hunslet	226	2·5	23·1	10·2	2·52
2. Park Square ..	242	2·7	32·2	13·3	2·77
3. University ..	297	3·3	35·6	11·9	1·99
4. Headingley Hill ..	449	5·0	51·8	11·5	1·98
5. Weetwood Lane ..	496	5·5	60·6	12·2	1·79
6. Garforth	395	4·4	49·0	12·4	1·88

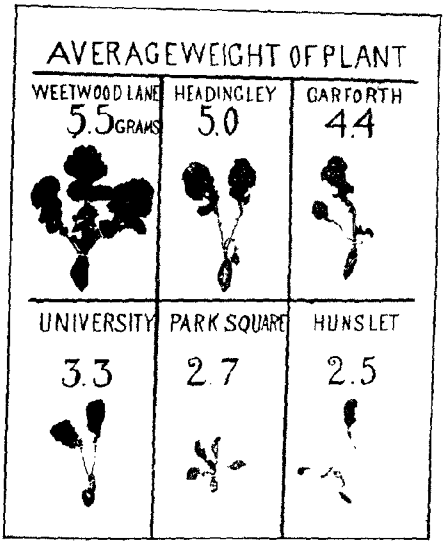

Fig. 1.

The most rapid germination took place at station 6 (Garforth) and the slowest at station 1 (Hunslet). One set of plants at Garforth was slightly damaged by chickens when the seeds were well through, but further damage was prevented. The plants were thinned out on May

30th, leaving 30 radish plants and 10 lettuce plants in each box. The radishes were finally lifted on June 30th, weighed, dried, re-weighed and the percentage of sulphur in the dry matter determined. The results are summarised in Table II and will be discussed later.

After the radishes had been lifted the lettuces were again thinned, leaving three plants in each box. These were finally removed on Sept. 19th, 1911 and gave the results summarised in Table III.

TABLE III. *Second Crop, Lettuces.* (Cp. Fig. 2.)

Station	Weight of crop		Percent. of SO_3 in dry matter
	Green gms.	*Dry matter* gms.	%
1. Hunslet	44	4·4	3·22
2. Park Square ..	56	5·3	2·61
3. University ..	104	10·2	1·99
4. Headingley Hill ..	120	11·4	2·02
5. Weetwood Lane	140	15·1	1·53
6. Garforth	175	18·2	1·20

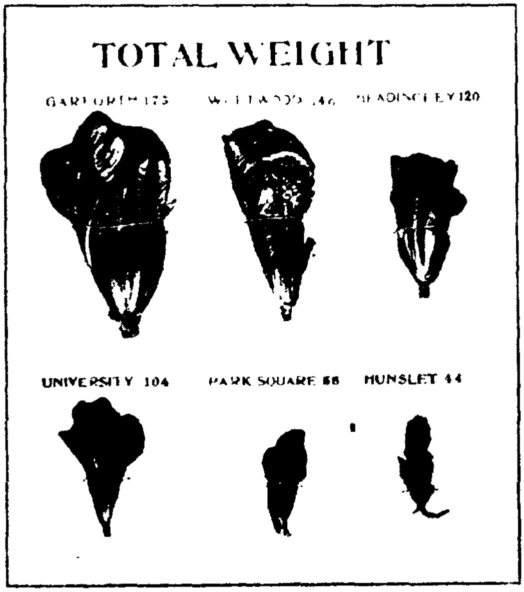

Fig. 2.

The lettuces were immediately followed by winter cabbage, three plants to each box. These did not do well at any centre, but the

differences between the different centres were very significant. Thus, at station 1 (Hunslet)—the most polluted centre—every plant had died off by the middle of November. By Christmas all were dead at stations 2 and 3; five plants were alive at station 4 and seven at station 6 (Garforth). Severe frosts in February 1912 proved fatal to all the plants at Garforth and to three plants at station 4 (Headingley). Only at station 5 (Weetwood Lane) did all the plants survive the winter (save for one that had suffered mechanical damage). At the remaining stations, out of 45 plants only two survived in March, these being at station 4—the station most nearly comparable in atmospheric cleanliness with station 5.

On March 20th, 1912, the remains of the winter cabbage were removed and replaced by fresh spring cabbage plants. These were grown on until August 15th, when they were cut and weighed etc., giving the results summarised in Table IV.

TABLE IV. *Fourth Crop, Cabbage.*

Station	Weight of crop		Percent. of SO_3 in dry matter
	Green gms.	*Dry matter* gms.	%
1. Hunslet	505	66	4·40
2. Park Square ..	1250	154	3·56
3. University ..	3056	365	3·09
4. Headingley Hill ..	4167	390	2·68
5. Weetwood Lane ..	3425	497	2·08
6. Garforth	1597	349	2·11

The cabbages were followed by wallflowers (Blood Red) planted out in the autumn of 1912 and left out until May 22nd, 1913. This crop, like the cabbage of the previous winter, furnished a demonstration of the inadvisability of autumn planting in smoke-infested areas. Nine plants were set out at each station; eight fairly well-grown plants were subsequently lifted alive at Weetwood (station 5), six each at Garforth (station 6) and Headingley (station 4), but at the other centres the plants that survived had made little or no growth, and they amounted to three only at station 3, one at station 2 and two plants at station 1[1].

[1] These results were borne out by the further experience at station 1 during the same winter with over 200 wallflower plants and 60 violas, all well established plants, set out in the soil of the garden in which our experimental boxes were sunk. Of these plants not a single wallflower and only two violas survived the winter.

The wallflower plants from the various stations showed very characteristic differences in root-development. Those grown in the most polluted areas were marked by an almost entire absence of root hairs and fibrous roots, in many cases nothing being developed but one long tap root (cp. Fig. 3). This feature we find to be still more pronounced in the case of plants grown in the native soil at each station, this having been exposed to the smoke pollution for many years.

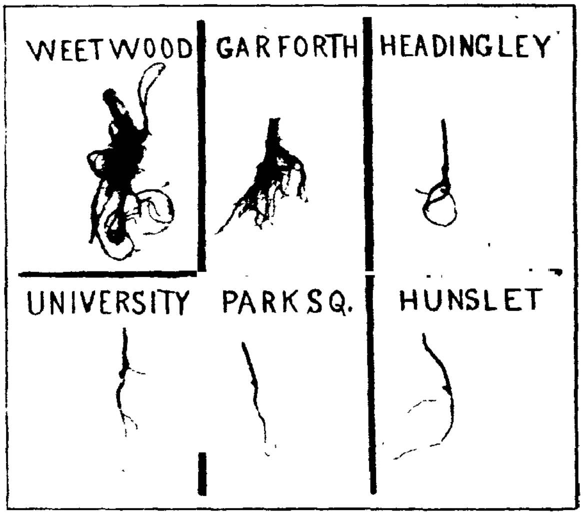

Fig. 3.

Having completed the record of the tests made with five successive crops we may now turn to consider more closely the results obtained. For this purpose the results for the three crops that were successively grown (1st, 2nd and 4th) are summarised in Table V, where in order to facilitate comparison the results obtained at station 5 (Weetwood)—the cleanest of the Leeds stations—are taken as 100 in each case.

Apart from a few slight irregularities the results indicate a fairly close correlation between the relative degree of purity of the atmosphere in the neighbourhood of the stations, as assessed from our earlier observations, and the actual amount of plant growth obtainable. The correlation is further shown with the accumulation of sulphur in the plants[1]. There is evidence, moreover, that the effects are cumulative, the Hunslet results becoming worse with each succeeding crop.

[1] Cf. Crowther and Steuart, This *Journal*, v. p. 405.

TABLE V.

Station	Relative purity of air as measured by freedom from sulphur (Table I)	Relative weight of crop. (Station 5 = 100)			Relative richness of dry matter in sulphur. (Station 5 = 100)		
		1st crop, Radishes	2nd crop, Lettuce	4th crop, Cabbage	1st crop, Radishes	2nd crop, Lettuce	4th crop, Cabbage
1. Hunslet	34	46	31	15	142	210	211
2. Park Square	37	49	40	37	156	171	171
3. University	55	60	74	89	112	130	148
4. Headingley	70	90	86	122	111	132	129
5. Weetwood	100	100	100	100	100	100	100
6. Garforth	80	80	125	47	106	78	101

It was of interest at the close of the vegetation tests, after the soil had been exposed at each centre for three years, to compare the effects, if any, of the varying pollution of atmospheric origin, upon the properties of the soil, more especially with regard to its content of calcium carbonate and its bacteriological qualities. Accordingly samples of the soil at each station—originally, it should be remembered, all drawn from the same bulk—were taken in the autumn of 1913 and examined. The results are summarised in Table VI.

TABLE VI.

Station	Calcium Carbonate in soil	Nitrogen as Nitrates	Total No. of Bacteria per gram of dry soil	Ammonia produced from peptone	Ammonia converted into nitrates	Nitrogen Fixed per gram of mannite
	%	pts per million	thousands	mgms.	mgms.	mgms.
1. Hunslet	0·12	1·2	876	64	1·2	15
2. Park Square	0·17	0·8	798	67	1·9	18
3. University	0·19	1·9	1054	78	4·3	19
4. Headingley	0·26	3·4	1236	88	6·4	21
5. Weetwood	0·30	4·6	1536	105	8·7	26
6. Garforth	0·34	5·1	1420	95	10·6	23

These results indicate clearly that the detrimental effect of the smoky atmosphere upon plant-growth—especially at stations 1 and 2—is partly due to unfavourable changes in the soil—such as the steady depletion of the stock of calcium carbonate and the inhibition of the activities of the nitrogen-adapting bacteria.

Certain other aspects of the injury to plants, especially the effects upon the activity of certain classes of enzymes in the plant, are at present under investigation, and will form the subject of a future communication.

Conclusion.

In submitting the results summarised in the foregoing pages we profess only to have demonstrated that in an industrial city such as Leeds smoke pollution is so concentrated or so persistent as to produce an immediate and measurable effect upon the growth of plants.

We are fully aware that in making our comparisons we have eliminated only one disturbing factor, that of soil differences. Our results are undoubtedly affected to some extent by other differences between the various stations such as altitude and exposure. These it was obviously impossible to equalise more than very roughly. Still the differences in plant growth observed are, in the extreme cases, so great and the gradation in passing away from the highly polluted centres is so clearly defined as to leave little doubt that the dominant factor in bringing about these effects was the variation in the quality of the atmosphere.

In conclusion, our thanks are due to T. Pridgin Teale, Esq., F.R.S., J. H. Wicksteed, Esq., M.I.C.E., and T. Goodman, Esq. for permission to carry out the experiments in their gardens, and for the close interest taken by them in the progress of the work.

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FURTHER STUDIES OF THE EFFECTS OF SMOKE FROM TOWNS UPON VEGETATION IN THE SURROUNDING AREAS.

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In two previous communications a summary has been given of the results obtained in determinations of the relative degree of atmospheric pollution, firstly in various parts of the city of Leeds[1], and secondly in the surrounding semi-urban and rural areas, to a distance in some directions of seven miles from the centre of the city[2]. The results obtained in the latter series indicated clearly the presence of extensive atmospheric pollution, showing the characteristics of coal smoke, in all parts of the area investigated. The degree of pollution was found to fall rapidly on passing in a northerly direction from the centre of the city into an area free from smoke-producing industries, but less rapidly on passing into similar areas to the north-east and east of the city, owing to the greater dispersion of the city smoke in these directions by the prevailing winds. On the opposite side of the city, from north-west round by south to south-east, smoke pollution was found to be very high in all quarters.

On the completion of this preliminary diagnosis it was decided to attempt to measure directly the effects of the atmospheric pollution upon crops grown on agricultural land in various parts of the area investigated. The method proposed was to grow the same crops at each station in the same soil placed there for the purpose, and to compare the weight and composition of the crops obtained.

Similar experiments upon the same lines, but upon a smaller scale, were already in progress within the city[3] and the preliminary results

[1] Crowther and Ruston. This *Journal*, iv. 25.
[2] Crowther and Steuart. This *Journal*, v. 391.
[3] Crowther and Ruston. This vol. p. 387.

obtained seemed to indicate that we might reasonably expect to get measurable differences in the yield of crops.

Accordingly seven experimental stations were selected, six being situated on the cleaner side of Leeds and the seventh, to give a marked contrast, on the more polluted side. The six cleaner stations were situated in pairs to the north, north-east and east of the centre of the city, there being in each direction a station at four miles and a second at seven miles from the centre. The seventh station was situated four miles out to the south-west, in the middle of a farm, with the nearest large source of smoke about a mile away. This station was about a mile distant from the one in this district at which samples of rain had been previously collected for twelve months[1], so that it was deemed advisable to remove the rain-collecting outfit to the site of the vegetation tests and continue the collection of samples there for a further 12 months (July 1, 1912—June 30, 1913). For purposes of comparison the collection was also continued for the same period at station E 7[2] (Garforth)—this being selected as the most convenient. The sulphur-content of the 12 months' rainfall, expressed as pounds of SO_3 per acre, is set out below, along with the corresponding totals obtained during the preceding 12 months:

Precipitation of Sulphur (lb. SO_3 per acre).

Station	1911–12	1912–13
	lbs.	lbs.
E 7	168	60
SW 3	268	
SW 4		352

The marked reduction in the Garforth (E 7) total was probably attributable to the great difference in character of the two periods—the earlier being notoriously wet, whilst the later included many prolonged spells of drought. It is all the more striking therefore to find on the other side an actual increase over the previous year's record, so that there could be no doubt as to the high degree of pollution of the atmosphere over this area. In view of the close network of industrial towns with which this part of the West Riding is covered it is indeed inevitable that such should be the case.

[1] Crowther and Steuart, *loc. cit.* p. 395, station SW 3.

[2] Station seven miles east of Leeds. This form of notation was used previously and is used here to indicate the relative positions of the experimental stations with regard to the centre of Leeds.

For the purposes of the crop tests about five tons of poor soil—practically subsoil—was taken from a small area in one of the fields at the Experimental Farm, Garforth, riddled, well mixed, and then used for filling seventy wooden boxes, each 1 sq. foot in cross-section and $1\frac{1}{2}$ ft. deep. These were then distributed, ten boxes to each station and were there sunk in arable land and fenced round with wire netting.

The stations had been previously selected with a view to obtaining sites that were comparable in exposure, elevation, etc.—a task of no small difficulty, and so far as elevation is concerned, admitting of only very rough approximation, the extreme range being from about 200 feet (E 7) to 400 ft. (N 4). Further inequalities were met with in the nature of the subsoil in which the boxes were sunk, that at station E 4 being the best, whilst at E 7, NE 4, NE 7 and SW 4 little fault could be found, but at N 4 it was peaty and at N 7 clayey. These differences undoubtedly affected the results.

On April 22 and 23, 1912, five boxes at each station were sown with perennial rye-grass (0·3 gm. seed in each box) and the remaining five with barley,—the grass and barley boxes alternating in position. Both crops germinated well at all centres, and later on the barley was thinned out to 28 plants per box. Fair growth was made in all cases, and the barley was cut on Sept. 18 and the rye-grass on Oct. 4. It had become clear before this, however, that the results would be of little or no value for the purposes of the experiment, owing to the disturbing influence of two factors—the excessive wetness of the season and the varying degrees of shelter afforded by the surrounding crop in the field. Thus the crops at N 5 and N 7, which received the benefit of the cleanest atmosphere, were hampered continually by the waterlogged condition of the boxes: and on the other hand the crops in the dirty district, SW 4, benefited by abundant shelter provided by the adjacent crops, wheat and rhubarb.

Climatic irregularities we could not control, but shelter effects we endeavoured to eliminate for the following season by removing the boxes, during the winter of 1912—13 to neighbouring pasture-fields at each centre. Here the boxes were sunk again, surrounded and covered over with wire-netting to keep out birds, rabbits, etc., and the whole further surrounded by a stout two-rail wooden fence to ward off grazing stock. Throughout the ensuing summer the grass inside these fences was periodically cut

The rye-grass sown in the previous spring survived the winter satisfactorily and was allowed to grow on. The alternate five boxes which had previously grown barley were again sown with barley in

April 1913, but owing to a sudden break in the weather before all the boxes had been sown the braird was so uneven that the plants had to be removed. It being then too late to re-sow barley, buckwheat was sown in these boxes on May 27 and 28—this crop having the reputation of being sensitive to smoke. Later the braird was thinned out leaving an equal number of plants in each box. Apart from weeding the boxes were not interfered with in any way until the crop was taken.

The season was in marked contrast to the preceding one, long spells of drought rendering the growth of the plants at times somewhat precarious, and leading eventually to the harvesting of the buckwheat a little prematurely on August 11 and 12.

Two cuttings of rye-grass were taken, the first on June 16 and 17 and the second on Oct. 27 and 28. At station NE 7 the rye-grass results were ruined by the persistent drifting of dead oak leaves from neighbouring trees into the boxes, causing the grass to be largely rotted out. For this reason the rye-grass results at this station have been discarded.

The results are summarised in the following tables :

Season 1913. *Buckwheat.*

Station	Yield of dry matter per box			
	Max.	Min.	Average	
	gms.	gms.	gms.	Relative to N 7 = 100
Agricultural area—N 7	26·4	23·4	24·5 ± ·4	100
N 4	24·5	19·8	22·6 ± ·5	92
NE 7	26·3	19·5	23·2 ± ·8	95
NE 4	26·3	22·8	24·8 ± ·4	101
E 7	25·0	18·1	21·9 ± ·8	91
E 4	26·1	22·5	24·6 ± ·5	100
Industrial area—SW 4	22·6	15·9	18·8 ± ·8	77
	Rye-Grass, 1st Cutting.			
Agricultural area—N 7	63·5	36·8	46·1 ± 3·2	100
N 4	40·8	30·6	35·8 ± 1·3	78
NE 7	rotted out			
NE 4	50·1	40·2	44·9 ± 1·3	97
E 7	44·2	29·7	38·0 ± 1·0	82
E 4	54·5	41·9	48·2 ± 1·5	104
Industrial area—SW 4	36·2	24·5	31·2 ± 1·5	68

One cannot attach much importance to the results of a single season's trial, but as we find ourselves precluded from continuing the tests it is necessary to summarise here the general indications of the above results.

It may first be noted that, so far as the seven-mile stations are concerned, the crop results as they stand are in substantial agreement with the records of smoke pollution previously obtained in these districts, but the differences are little, if at all, outside the probable range of error.

At the four-mile stations, however, except for the marked inferiority of the results at the "dirty" station, SW 4, there is no evidence of differences parallel with those found in the rain records.

We are of opinion that the explanation of this discrepancy probably lies partly in the character of the soil in which the boxes were embedded, and partly in differences of elevation, aspect, wind-breaks, etc.

It has already been pointed out that station E 4 was especially favourably situated as regards soil, but station N 4 was probably handicapped—although the site used in 1913 was much dryer than that used in the previous year. It is possible also that the proximity of peat to the boxes at this station in the earlier year may have established in the boxes detrimental conditions that were not eliminated during the following season.

In any case it was hardly to be expected that any sharply defined grading of the stations would be obtained in the course of one or two seasons.

When we come to the comparison of the crop obtained on the more polluted side of the city at station SW 4, with those obtained in the cleaner areas on the other side the difference is quite sharply defined. If we group together the results on the cleaner side we get the following comparison :

Average Crops per Box.

	Buckwheat	Rye grass	
		1st crop	2nd crop
	gms.	gms.	gms.
Clean districts	23·6±·3	42·6±1·0	13·6
Dirty district	18·8±·8	31·2±1·5	10·8
Difference	4·8±·9	11·4±1·8	2·8
Representing a nominal loss of crop in the dirty district of	20 %	27 %	21 %

It would have increased the interest and value of the test if more centres on the polluted side of the city could have been included, but difficulties of supervision rendered this practically impossible. The station selected is typical, however, of very large areas of the West Riding that are being utilised for agriculture.

The crops at this centre were carefully examined at intervals for external signs of smoke damage, but beyond a slight reddening of the leaf-tips of the rye-grass no visible symptoms could be detected. At Garforth there were less marked effects on rye-grass and at several places also on buckwheat before cutting but this may have been due to drought. In passing it may be remarked that the season of 1913 was regarded by gardeners in and around Leeds as a particularly good one, so far as freedom from obvious smoke damage was concerned.

Buckwheat (Whole Plants).

	Station N 7	Station E 7	Station SW 4
	%	%	%
Crude protein	7·7	8·8	9·3
Ether extract	2·1	2·0	2·5
Crude fibre	25·4	24·4	24·9
Ash	6·5	7·4	8·5
N-free extractives (by difference)	58·3	57·4	54·8
Total SO_3	0·49	0·61	0·88
Water-soluble SO_3 ..	0·20	0·29	0·52
"Basicity" of ash[1] ..	85	77	70

Rye-Grass, 1st Cutting.

	Station N 7	Station E 7	Station SW 4
Crude protein ..	5·9	6·9	6·9
Ether extract	2·0	2·2	2·0
Crude fibre	33·3	34·9	35·5
Ash	6·7	7·3	6·9
N-free extractives (by difference)	52·1	48·7	48·7
Total SO_3	·94	1·05	1·40
Water-soluble SO_3 ..	·60	·59	·66
Basicity of Ash[1] ..	15	12	11

[1] Gms. H_2SO_4 required to neutralise 100 gms. ash.

In previous experiments[1] appreciable differences were found in the composition of Timothy grass grown on soils watered with neutral and acid rain-waters. For the purposes of comparison the crops grown in the present tests at three of the stations were analysed viz. at station N 7 (cleanest station), E 7 (intermediate in cleanliness) and SW 4 (dirtiest). The results are given on p. 400.

The results show no appreciable difference in the ingredients determinative of nutritive value, but the differences in sulphur-content which have been pointed out previously[2] as characteristic of smoke-pollution are clearly defined. It will be noted further that the ash of the plants grown in the dirty district was distinctly more acid in character than in the case of the cleaner crops.

Effects upon soil.

After about two years' exposure in the boxes the soil at stations N 7 and SW 3 was sampled for the purpose of determinations of lime-content and for bacteriological examination.

Calcium carbonate was estimated by Amos' method, with the following results :

Station	N 7	0·13 %.
,,	SW 4	0·11 %.

The difference indicated cannot be regarded as outside the range of combined errors of sampling and analysis.

The samples of soil taken with the usual precautions for bacteriological examination were employed to ascertain the numbers of bacteria that would grow on agar plates of —5 acidity[3].

Counts done in triplicate at the end of ten days' incubation at about 20° C. gave the following results in millions per gm. of soil :

Station	N 7	$29{\cdot}4 \pm 1{\cdot}6$	Difference
,,	SW 4	$22{\cdot}9 \pm 3{\cdot}8$	$6{\cdot}5 \pm 4{\cdot}0$.

A difference is indicated in favour of the cleaner district, but there is no great degree of certainty that it is not purely accidental. From the *general appearance* of the plates there could be little doubt as to the more vigorous growth obtained with the soil from N 7.

[1] Crowther and Ruston, *loc. cit.*

[2] Crowther and Steuart, *loc. cit.*

[3] i.e. in making the medium 5 c.c. of normal sodium carbonate solution were added to 1 litre of neutralised medium.

General Observations.

The systematic observations of trees and general vegetation referred to in our earlier paper have been continued and extended.

The more striking features are summarised below :

Trees (1913).

In the earlier observations no general effect upon the opening of the buds of trees was detected, but closer observation has revealed that individual buds may be so badly injured that they either open quite late in the spring or not at all.

Leaves may be injured at any stage from the opening of the buds onwards, but they seem to be most susceptible when just expanding. If the leaves are injured for several seasons in succession the trees naturally get sickly and die off. First isolated twigs die, then the tops and branches, and finally the whole tree goes.

During May acute damage appeared on the leaves in the dirtier districts. For example, on May 8th near station SW 4 many of the young oak leaves peeping out from the buds were completely blackened, shrivelled up and dead. On a large open space (Woodhouse Moor) about a mile to the north of the centre of Leeds, sycamores attempted to produce new leaves throughout the season, but all were damaged severely so that little recovery was possible.

Towards the beginning of September in many parts of the town horse-chestnuts had only the ribs of their first set of leaves left, but these had been augmented by a complete set of new green leaves.

In woods three miles to the south (Middleton) the smoke blew through practically unchecked during May and considerable damage was done. As the wood got covered in with foliage little fresh damage had been done by July 11th. Elders which were previously blackened and unsightly had made apparently a complete recovery ; only when the branches were pushed aside could signs of the damaged shoots be discovered. Young oaks had not made a good recovery and the old damage was still evident ; the red patches of dead leaf-tissue had fallen away leaving fresh green laminae full of holes ; where the opening buds had been destroyed completely the twigs were still bare. The same was the case with the damaged leaves of other trees.

Conifers are more susceptible than deciduous trees, and in general trees, particularly when isolated, more so than most farm crops.

Farming.

In the smoky districts farmers complain that they have to "apply heavier tillage" to get good results. It is quite common to apply 30 tons of dung to the root crop. Heavier feeding of stock is necessary, and even then the results may be unsatisfactory[1]. Hedges are bad and expensive to replace. Wire fencings last one-half or one-third of the time they ought to. Galvanised netting lasts only four or five years. Corrugated iron roofing on sheds has also a very short life. In the pastures[2] the "finer grasses die out and they become coarse and distasteful to stock." In extreme cases the grass dies out in patches in which only sorrel will live, and applications of lime are then found greatly to improve matters. In some cases clovers, specially red clover, die out during the winter and the farmers are consequently leaving clovers out of their one-year leys and sowing only rye-grass.

An attempt was made to find out which grasses do best in smoky districts by examining meadows while the grasses were in flower. Cocksfoot, rye-grass, fescue grasses and meadow foxtail do quite well, and all the commonly sown meadow grasses. Too often weeds and soft brome grass predominated. Large clumps were often filled with Holcus or Agrostis, and sweet vernal occupied too much space in the poorer meadows. Red clover seemed quite common. The coarseness of the pastures may be due to the animals not caring to eat them close.

The young leaves of cereals may turn red at the points and possibly may bleach. The shoots are thinned out, the crop ripens unevenly, empty or half-filled ears occur and the pickles do not swell properly. Serious damage of this character, however, is limited to the near neighbourhood of coke ovens, forges, etc. All over the dirtier districts the grain is a dirty bleached grey colour and sells badly when exposed for sale in the market beside clean samples. A farmer may easily lose £1 per acre of white crop by this means alone. Red wheats show up the dirt less than white and are consequently favoured; black winter oats and "Tartar Kings" are the favourite varieties of oats on the farms in the dirtiest areas. Good malting barley cannot be grown.

The following are the results of some examinations of grain. Samples were obtained from farms on which obvious smoke damage was known to occur. They are compared with average samples, and show a considerable falling off in the pickle weight. The SO_3 figures were

[1] See Steuart, *Journal of the Board of Agriculture*, Jan. 1914, p. 897.

[2] Cf. Webster's "Town Planting," pp. 6 and 171.

obtained as follows: 25 gms. of grain were washed with small successive portions, amounting to 250 c.c. of distilled water. The washings were boiled with bromine water, evaporated down, and the sulphate precipitated in the usual way.

Description of grain	Wt. of 1000 corns in grams	SO_3 from 100 grams	Per cent. germination
Black oats grown near coke ovens ..	35·3	·028	96
Black oats grown on far side of field ..	38·9	·023	—
Clean sample black oats for seed ..	45·6	—	—
Black oats grown near colliery, etc. ..	39·3	—	97
Yielder oats grown near coke ovens ..	—	·032	—
White oats grown near vitriol works ..	—	·034	—
Clean sample oats from Ayrshire ..	—	·014	—
Barley grown near vitriol works ..	—	·032	—
Clean Scots sample of barley	—	·003	—
Clean East Lothian sample	—	·012	—
Barley from Manor Farm Garforth ..	51·8	—	99
Barley from near tile glazing works, etc.	32·7	·044	98
Wheat, average market sample	45·0	—	—
Wheat grown near vitriol works ..	30·7	—	—

Farmers and gardeners have often great difficulty in obtaining compensation for smoke and fume damage, particularly where the source of the noxious gases is complex. Acute smoke injury on farms is restricted in its range and it is rarely found that a number of crops grown successively in a given locality show this effect. The detrimental action of smoke is found at its maximum in damp weather under atmospheric conditions that cause an accumulation and concentration of the smoke gases over the growing crops. A farmer seldom can complain of a total loss of crop and it is this element of partial loss varying from year to year which makes the diagnosis of smoke damage and its assessment for purposes of compensation so hard to decide. In any case monetary compensation is little satisfaction to a real farmer. Hence many of the more enterprising farmers refuse to farm in smoke infested districts, while in other cases the men get very discouraged.

Gardeners with experience in clean districts state that "everything seems to stand still." Quick growing plants and plants with thick leaves seem to stand the smoke best. Smoke dirties everything. With cauliflowers and broccoli the practice is to break one or two leaves over the developing flower to keep off the dirt. A common complaint is that fruit trees and bushes do not thrive or bear well. In the worst

districts rhubarb for forcing is practically the only market crop grown in the open. "Winter onions, cabbage and lettuce have failed me so often that I have ceased to grow them ;—before the smoke was so bad crops used to stand the winter." This is a more or less general experience in smoky districts. Summer cabbages may be grown with fair success, while those sown in July and planted out in September die off during the winter. A similar effect is commonly experienced with young flower seedlings[1].

It is difficult to prepare a good lawn in some parts of the town as the finer grasses die out. The soil requires special preparation and dusting with chalk. Seed mixtures are consequently sold containing considerable quantities of agrostis, perennial rye-grass and even timothy.

In some parts roses have been tried over and over again but in a year or two nothing of them remains. Each gardener in a polluted district knows fairly well what varieties he can most safely propagate and he has to stick rigidly to these, without much choice in some cases. Thus in a cemetery garden on the more polluted side of Leeds mainly stocks and violas were relied on for a show of flowers and the violas died off during the winter. Slips for their propagation had to be obtained from a clean district.

These are some of the difficulties that beset the agriculturist and horticulturist in the neighbourhood of the large industrial city. But for smoke they might reap far greater benefit from the proximity of large bodies of consumers. We had hoped to continue our investigations until we could arrive at a reliable assessment of the material loss that is inflicted upon the farmer and gardener over thousands of acres of this country by the distribution of excessive smoke from the towns, but must rest content to have obtained sufficient preliminary evidence to warrant a further extension of the investigations when circumstances permit.

In conclusion we would express our indebtedness to a great number of farmers, gardeners and others who have assisted us in various ways and especially to those who have kindly given us facilities for our crop tests in their fields.

Further we would not omit to record our appreciation of the support accorded to us by the Board of Agriculture and Fisheries, out of the research funds at their disposal, without which the extension of our investigations into the rural areas would have been quite impossible.

[1] Compare this vol., p. 391.

THE ESTIMATION OF CARBOHYDRATES. IV.

THE PRESENCE OF FREE PENTOSES IN PLANT EXTRACTS AND THE INFLUENCE OF OTHER SUGARS ON THEIR ESTIMATION.

BY WILLIAM A. DAVIS AND GEORGE CONWORTH SAWYER.

(Rothamsted Experimental Station.)

PREVIOUS papers of this series have dealt with the estimation of the hexoses, disaccharides and starch present in plant material. In the present paper we bring forward evidence to show that free pentoses are also usually present in the alcoholic extracts, and have to be taken into account in the scheme of analysis[1]. The existence of free pentoses in plants has not hitherto been recognised; the evidence that they are actually present may briefly be summarised as follows.

Substances undoubtedly exist in these extracts which are soluble in 80 % alcohol, are not precipitated by basic lead acetate, are unfermentable by ordinary yeasts and exercise a certain reducing power after all other sugars have been fermented away. This reducing power (calculated as a mixture of arabinose and xylose) corresponds with a proportion of pentose very nearly identical with that calculated from the weight of phloroglucide obtained on subjecting the purified aqueous solution used in the analysis to the ordinary Kröber-Tollens distillation process. These facts taken together can only be explained by assuming that *free* pentoses are actually present and not by any assumption that the furfural obtained on distilling with acid originates from gums or pentosans or from the other sugars present.

It is true that, when cane sugar or certain hexoses are distilled with hydrochloric acid, small quantities of furfural-like compounds are formed which yield an insoluble phloroglucide and that, in consequence, the estimation of *small* quantities of pentose in presence of large

[1] *J. Agric. Science*, 1913, v. 465.

quantities of these sugars is not strictly accurate. But we show below that the proportion of other sugars present in such extracts as we have dealt with can give rise only to a relatively small proportion of the phloroglucide actually found and does not materially interfere with the pentose figure obtained by the ordinary process.

The occurrence of pentoses in plants would explain the accumulation of free pentoses in the "*vinasses*" remaining after distilling off the alcohol from the fermented liquors of distilleries which make use of the molasses of the beet-root sugar industry[1]. Other sugars are completely fermented away by the ordinary distillery yeasts but, as we have found, the pentoses remain completely unfermented, and therefore form a very large proportion of the total carbohydrates existing in these liquors after the fermentation is complete.

Presence of Pentoses in Alcoholic Extracts of Foliage Leaves.

In a large number of estimations made with the leaves of different plants (mangolds, turnips, *Tropaeolum majus*, *Helianthus*, carrot, potato, etc.) the percentage of pentose obtained by the distillation method, when applied to the alcoholic extract treated according to our scheme of analysis, was found to range from 0·3 to 1 %, calculated on the total vacuum-dried matter. The fresh leaf material (about 1000 grms.) used in our analyses was dropped into boiling alcohol (2 litres) containing a little ammonia to stop enzyme action, and was then extracted for 18 hours in a large specially constructed metal form of Soxhlet extractor. After the operations outlined in our scheme[2] 50 c.c. of the 2 litres of solution A were distilled with acid according to the Kröber-Tollens method. The phloroglucide frequently weighed from 0·015 to 0·026 grm.; thus, for instance, in one case (*Mangold Leaf*, 1 a.m. Oct. 12, 1912) when the total pentose was 0·78 %, the phloroglucide weighed 0·0192 grm. Now the total sugars in this case did not exceed 20 grms. in the 2 litres of solution, so that the amount of sugars other than pentoses in the 50 c.c. used for the analysis did not exceed 0·5 grm. We have found (see p. 411) that 0·5 grm. of carefully purified cane sugar, dextrose or maltose, when subjected to Kröber's distillation process gives only 0·0036 to 0·0047 grm. phloroglucide, so that although it is perfectly true, as contended by Kluyver[3], that the

[1] Matignon, *Bull. Soc. d'Encouragement*, 1914, CXXI. 445.

[2] *loc. cit.* p. 466.

[3] *Biochemische Suikerbepalingen*, Leiden, 1914, pp. 181 and 190.

hexoses etc. are a source of error when estimating small quantities of pentose, their presence does not account for the greater part of the phloroglucide actually obtained. We show below (p. 411) that when 0·01 gram of pure arabinose is mixed with 25 times its weight of cane sugar (0·25 grm., a proportion of total sugar to pentose far greater than usually occurs in these extracts) the weight of phloroglucide obtained being 0·0094 grm., the result obtained was about 20 % higher than in the absence of cane sugar (0·0162 grm. arabinose, as compared with 0·0128 grm.). When 0·02 grm. arabinose is distilled with 0·25 grm. cane sugar (a proportion corresponding with that usually found in our extracts) so that 0·018 grm. phloroglucide is weighed, the error in the pentose estimation is smaller, the result being about 15 % high (0·0256 grm. found instead of 0·0222).

That *free* pentoses actually exist in these alcoholic extracts is also borne out by the following facts. When portions of the aqueous solution A of our scheme are fermented as completely as possible, and the reducing power of the fermented solutions estimated after purification with alumina cream, the residual reducing power agrees fairly closely with that calculated for the proportion of pentoses present, as determined by the ordinary distillation method. This residual reducing power can indeed be used as a means of estimating the pentoses present, as the following example shows:

Turnip Leaves, July 9, 1913.

The pentose found by distilling 50 c.c. of solution A = 0·60 % on the total dry matter.

One litre of solution A was then evaporated *in vacuo* to about 175 c.c. and made up to 250 c.c.

Five portions of 50 c.c. (each representing 200 c.c. of the original solution A) were then sterilised, and fermented as completely as possible for three to five weeks with a pure culture of baker's yeast[1]. 5 c.c. of alumina cream was then added to each portion and the solution filtered and washed to 100 c.c. with boiling water, the volume being finally made up to 100 c.c. at 15°. 50 c.c. of the filtrate of each (=100 c.c. of the original solution A) was then used to ascertain the reducing power.

[1] A large number of experiments, details of which will be published later, have shown that baker's yeast does not ferment or assimilate the pentoses (xylose and arabinose); on the other hand the maltase-free yeasts (*S. Marxianus*, *S. exiguus* and *S. anomalus*) gradually but slowly destroy these sugars.

The average value for the CuO found = 0·0512 grm. Now if the whole of the reducing power is due to pentoses, assuming the mean value found for arabinose and xylose under these conditions[1] we have:

Percentage of pentose calculated on the total dry matter

$$= \frac{0{\cdot}0512}{2{\cdot}53} \times \frac{2000}{100} \times \frac{500}{440} \times \frac{100}{89{\cdot}4} = \mathbf{0{\cdot}51}\ \%.$$

There is thus a fairly close agreement between the result obtained for pentose by direct distillation and that obtained on the assumption that the slight residual reducing power which remains after fermentation is due solely to pentoses. It is true that the result obtained by the direct distillation by the Kröber-Tollens method (0·60 %) is slightly higher than the other value (0·51 %) but the difference is exactly of the order (18 % higher) that would be expected owing to the production of phloroglucide from the sugars other than pentoses present in solution A. The value 0·51 % probably more correctly gives the true proportion of pentoses present, but for practical purposes the difference between 0·5 and 0·6 % is of little importance, as the error introduced in correcting the calculation for the reducing sugars present by such a difference does not exceed 1 milligram of CuO. For most purposes therefore little error is introduced by estimating the pentoses by the distillation process, but when it is desired to estimate the pentoses present with the highest possible degree of accuracy the process suggested by Kluyver may be employed: the other sugars (dextrose, laevulose. cane sugar, etc.) should be completely fermented away with *S. cerevisiae* and the pentose determination carried out on the fermented material, after adding alumina cream and diluting to a known volume.

From the above example with turnip leaves it appears that in the case of alcoholic leaf extracts prepared by our method the slight residual reducing power which always remains after complete fermentation of the solution with yeast is due to the pentoses present. In nearly 400 fermentation experiments made with extracts of leaves of many different kinds, the amount of this residual reducing power has been found to be proportional to the pentoses present as estimated by the Kröber method, so that it is highly probable that it is to be attributed solely to free pentoses.

[1] Daish, *J. Agric. Sci.* 1914, VI. 255.

Effect of other Sugars on the Kröber-Tollens Method of Estimating Pentoses.

It has been generally recognised since the early days of this method that the ordinary carbohydrates such as cane sugar, galactose and dextrose give small quantities of furfural-like substances[1] when subjected to distillation with hydrochloric acid[2], but the amounts are, for practical purposes, so small that they can generally be disregarded[3]. Stoklasa[4] states that 100 grms. of cane sugar gives about 0·22 grm. of furfural, and Kröber and Rimbach[5] give this figure as about 0·5 grm. In the ordinary estimation of pentoses and pentosans these amounts can generally be disregarded, but when very small amounts of pentose have to be estimated accurately, Kluyver's experiments and our own show that a considerable error may be introduced owing to the presence of excess of hexose sugars; in such cases, the pentose should be estimated after completely fermenting away the other sugars by ordinary yeast. As a rule, however, in the scheme of analysis we have adopted, such a refinement is unnecessary as such differences, as, for example, between 0·5 and 0·6 % of pentose, do not materially interfere in calculating the results obtained for the other sugars.

Experiments with pure sugars.

The sugar was dissolved in 50 c.c. of water and subjected to Kröber's distillation process in Jena glass flasks fitted with rubber stoppers[6].

[1] It is probable that these sugars give, not furfural, but hydroxymethylfurfural, which also forms a sparingly soluble phloroglucide. When these sugars are distilled with acid, there is a marked difference between the colour changes shown on adding the phloroglucide from those given with pure arabinose, or pure furfural. In the latter cases, the solution is first yellow and then turns green. With the hexoses, the solution changes from yellow to a *claret red* and then finally to green. With mixtures of pure arabinose and hexoses the claret colour is always observed. [*Note added Oct.* 16, 1914. Since this paper was written, Cunningham and Dorée in a communication to the *Biochemical Journal*, 1914, VIII. 438, dated July 13, have dealt with several of the points raised above somewhat more fully than we have done; they give qualitative evidence that hydroxymethylfurfural is actually formed when hexoses are distilled with hydrochloric acid under the Kröber-Tollens conditions.]

[2] Compare Gunther, *Gött. Dissert.* 1891, p. 19.

[3] Cp. Kruger, *Rostock Dissert.* 1895, p. 29.

[4] *Zeit. Zuckerind Böhmen*, XXIII. 291.

[5] *Zeit. angew. Chem.* 1902, 508.

[6] A necessary precaution in the estimation of pentoses and pentosans which is not emphasized in the standard text-books is that the distillation flasks should be fitted with rubber stoppers and *not* with ordinary corks. The latter are invariably attacked by the

Pure hexoses (several times recrystallised).

0·5 grm. maltose (free from dextrin, etc.), weight of phloroglucide = 0·0036 grm.

0·5 grm. dextrose, weight of phloroglucide = 0·0040 grm.

0·5 grm. cane sugar, weight of phloroglucide = 0·0047 grm.

0·01 *grm. arabinose* (specially prepared from gum arabic and carefully purified by several crystallisations).

1. Gave 0·0068 grm. phloroglucide.
2. Gave 0·0060 grm. phloroglucide.

Average 0·0064 grm. phloroglucide = **0·0129** grm. pure arabinose.

0·01 *grm. arabinose* + 0·25 *grm. cane sugar.*

1. Gave 0·0097 phloroglucide.
2. Gave 0·0090 phloroglucide.

Average 0·0094 grm. phloroglucide = **0·0162** grm. arabinose.

Here the addition of 0·25 grm. cane sugar has increased the yield of phloroglucide by 0·0030 grm. and the apparent pentose by almost 25 %.

0·02 *grm. arabinose alone.*

1. Gave 0·0150 grm. phloroglucide.
2. Gave 0·0146 grm. phloroglucide.

Average 0·0148 grm. phloroglucide = **0·0222** grm. arabinose.

0·02 *grm. arabinose* + 0·25 *grm. cane sugar.*

1. Gave 0·0175 grm. phloroglucide.
2. Gave 0·0185 grm. phloroglucide.
3. Gave 0·0178 grm. phloroglucide.

Average 0·0179 phloroglucide = **0·0256** grm. arabinose.

Here the cane sugar (0·25 grm.) has caused an increase of 0·0031 grm. on the phloroglucide and an increase of 16 % on the pentose.

With larger quantities (0·2 grm.) of arabinose, cane sugar or dextrose still give rise to an increase in the phloroglucide of about 2 milligrams, but the error in the pentose estimation is diminished proportionately to the larger quantity of arabinose and becomes approximately 1 % on the pentose present.

hydrochloric acid and give appreciable quantities of furfural. The following example shows this. A series of "blanks" was carried out with hydrochloric acid in a flask fitted with ordinary corks. The results were as follows: 1st distillation gave 0·0022 phloroglucide; 2nd distillation gave 0·0042; 3rd distillation 0·0045; 4th 0·0035; 5th distillation gave 0·0013 grm. phloroglucide. When similar distillations were made in a flask fitted with rubber stoppers there was no change of colour after adding the phloroglucinol and the weight of phloroglucide obtained was *nil*.

Arabinose alone.

Taken 20 c.c. of an approximately 1 % solution of arabinose.

1. Gave 0·1716 phloroglucide.
2. Gave 0·1726 phloroglucide.
3. Gave 0·1705 phloroglucide.

Average 0·1716 phloroglucide = **0·1964** grm. arabinose.

20 *c.c. of the same solution* + 0·2 *grm. cane sugar.*

Phloroglucide weighed 0·1735 grm. = **0·1985** grm. arabinose.

20 *c.c. of the same solution* + 0·2 *grm. dextrose.*

Phloroglucide weighed 0·1735 grm. = **0·1985** grm. arabinose.

Discussion.

The results given above can be explained only by the assumption that free pentoses are present in the leaf-extracts we have examined. Kluyver[1] in discussing our first paper[2] suggested that pentoses are absent in such cases and that their estimation by the Kröber-Tollens method may give rise to considerable error owing to the production of furfural-like compounds from the other sugars present. We have shown above that, although some error does arise from this cause, it is not significant in the majority of cases. Kluyver emphasized the fact that we had merely stated in our previous paper that pentoses are present without giving any experimental evidence. This we have now done.

SUMMARY.

Evidence is brought forward to show that free pentoses exist in the alcoholic extracts of foliage leaves of different plants. Their amount can be estimated with a fair degree of accuracy by the ordinary distillation process or by the reducing power of the purified liquor after other sugars have been fermented away.

When, however, small amounts of pentose have to be estimated accurately in presence of large quantities of other sugars, it is advisable, as suggested by Kluyver, to ferment away these sugars before applying Kröber's process.

[1] *loc. cit.*

[2] Davis and Daish, *J. Agric. Sci.* 1913, v. 465.

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THE HYDROLYSIS OF MALTOSE BY HYDROCHLORIC ACID UNDER THE HERZFELD CONDITIONS OF INVERSION.

A REPLY TO A. J. KLUYVER.

By WILLIAM A. DAVIS.

(Rothamsted Experimental Station.)

In a recently published important monograph A. J. Kluyver[1] has independently extended to other sugars the principle adopted by the writer in a recent paper[2] for estimating maltose—namely the use of special yeasts, capable only of fermenting certain individual sugars. Whilst, in general, Kluyver supports the views we have put forward, he considers it necessary to make an "earnest protest" against our statement that, during the inversion of cane sugar by hydrochloric acid under Herzfeld conditions, there is danger of maltose undergoing hydrolysis. He refers to the work of Jalowetz[3] which, he considers, made it clear that under these conditions the maltose remains unchanged and gives additional experiments of his own to prove the same point.

He states, moreover, that the evidence we have brought forward is "extremely misleading" in so far as it was hardly possible to conclude from our experiments, which showed the effect of heating maltose solutions with hydrochloric acid during two to six hours, that any perceptible decomposition occurred during so short a time as is usual in the Herzfeld conditions of inversion.

It is quite true that in our paper we gave only the data obtained by heating 1 % solutions of maltose with 2·44 % hydrochloric acid at 70° during periods ranging from two hours to 24 hours, the decomposition of maltose varying from 28·8 to 94 % of the total quantity. But

[1] *Biochemische Suikerbepalingen*, Boekhandl. E. J. Brill, Leiden, 1914, pp. 223.

[2] Davis and Daish, *J. Agric. Sci.* 1913, v. 437.

[3] *Zeit. angew. Chem.* 1895, 208.

we gave also the curve of hydrolysis for the total period and it is a simple matter, from the data given, to calculate that, during the entire period, the decomposition follows approximately the ordinary logarithmic law of mass action, viz. $k = \frac{1}{t} \log \frac{1}{1-x}$. During the first two hours, in which 28·8 % of the maltose is hydrolysed, if t be taken in hours and ordinary logarithms employed, $k = 0{\cdot}074$. Using this constant and calculating from the equation, the percentage of maltose decomposed in six minutes (0·1 hour) is 1·75 %[1].

Now working with 75 c.c. of a 1 % solution for maltose under the conditions we gave, diluting to 100 c.c. and using 25 c.c. of the solution to measure the cupric reducing power, it can easily be calculated that if 1·75 % of the maltose is converted to dextrose the actual weight of cupric oxide obtained under Brown, Morris and Millar conditions is nearly 4 milligrams greater than if no hydrolysis occurred: an amount, as will be seen below, by no means to be ignored.

The following experiments made with an approximately 1 % solution of carefully purified maltose, following Herzfeld's conditions exactly, confirm what is very clear from the considerations given above —namely, that marked hydrolysis of maltose actually occurs under these conditions.

Series A. 1. 75 c.c. of an approximately 1 % solution of maltose (= 0·7196 gram anhydrous maltose) were diluted to 100 c.c. at 15°; 25 c.c. portions used for the copper reducing power gave 0·2453 and 0·2456 grm. CuO, or an average value of **0·2455** grm. CuO (= 0·1799 grm. maltose).

2. To 75 c.c. of the same solution 5 c.c. of hydrochloric acid of sp. gr. 1·188 were gradually added and the mixture heated in a bath of water at 70°; 2½ minutes were required to raise the temperature of the mixture to 67° and the solution was then heated exactly five minutes longer and subsequently rapidly cooled. The thermometer was washed with water, the solution exactly neutralised with sodium hydroxide and diluted to 100 c.c. at 15°.

25 c.c. gave (in two experiments) an average value of **0·2522** grm. CuO.

Thus the treatment with hydrochloric acid has caused an increase in the CuO of more than 6 milligrams, which is even greater than the

[1] Under Herzfeld conditions about three minutes is taken to heat the solution to 68–70°, and subsequently the solution is heated exactly five minutes; the six minutes used in this calculation does not therefore unfairly represent the total time of heating at 70°.

milligrams theoretically required if 1·75 % of the maltose were hydrolysed.

Series B. 1. 75 c.c. of another solution of maltose (= 0·8096 grm. anhydrous maltose) were diluted to 100 c.c.

25 c.c. gave in three experiments an average of **0·2761** grm. CuO (= 0·2024 grm. maltose).

2. 75 c.c. of the same solution were treated with 5 c.c. of hydrochloric acid exactly under Herzfeld conditions (3 mins. heating to 67°, 5 mins. at 68—70°), neutralised and made to 100 c.c.

25 c.c. gave an average value **0·2802** grm. CuO. Here there is an increase of 4·1 milligrams of CuO caused by the hydrolysis of maltose, which agrees very closely with that calculated for the change of 1·75 % to dextrose.

Conclusions.

The foregoing experiments show that perceptible hydrolysis occurs when dilute solutions of maltose are heated with hydrochloric acid under Herzfeld conditions. They justify our former contention that it is preferable to estimate cane sugar in plant extracts when maltose or other glucosides are present by making use of citric acid under the conditions we prescribed, as we have shown that, under these conditions, no perceptible hydrolysis of maltose is to be feared, whilst the hydrolysis of cane sugar is complete. Owing to the exigencies of the method of analysis we outlined, all the determinations (of reducing sugars, cane sugar, maltose) have to be made on quantities representing the same volume of the original solution (25 c.c. of solution B in our scheme); consequently when the proportion of reducing sugars or maltose is high, the cane sugar has to be calculated from an increase in reducing power caused by inversion which corresponds with only about 0·1 gram CuO. An error of 4 to 5 milligrams CuO in such a case would represent an error of 4 to 5 % of the cane sugar present.

The fact that the slight hydrolysis of maltose which occurs under Herzfeld conditions was not detected by Kluyver or Jalowetz is probably owing to their method of estimating the reducing power not being sufficiently delicate for the purpose. In Kluyver's experiments, in which the copper remaining was estimated volumetrically by the iodometric method, 44·0 milligrams of maltose per c.c. were found before treatment with hydrochloric acid and 43·0 milligrams afterwards. Thus, instead of an actual increase in the apparent maltose caused by

this treatment there was a *loss* of nearly $2\frac{1}{2}$ % ; a clear indication that the method of analysis was not so accurate as is necessary in such a case.

Summary.

It is shown that contrary to the statements of Kluyver and others, maltose undergoes slight hydrolysis (to the extent of about 2 % when 1 % solutions of maltose are used) when heated with hydrochloric acid under Herzfeld conditions. It is preferable therefore to adopt 10 % citric acid, under the conditions formerly laid down, in estimating cane sugar in plant extracts when maltose is likely to be present.

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PARTIAL STERILISATION OF SOIL BY VOLATILE AND NON-VOLATILE ANTISEPTICS.

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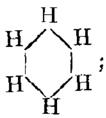
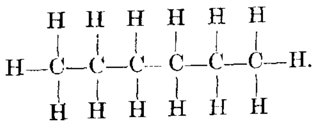
The experiments of Russell and Hutchinson and of other investigators have shown the general effects of partial sterilisation with volatile antiseptics and heat. In their experiments only a limited range of antiseptics was used—the most important being toluene, and this was selected as the most inert that could be found. The effect of this inert volatile antiseptic having been studied in some detail it becomes necessary to test a wider range of substances to see how far the phenomena are general. This has been done in the following paper. In order to set some limits to the enquiry attention has been confined to simple compounds which volatilise or decompose without much difficulty, and benzene was made the starting-point owing to its similarity to toluene which has been pretty completely studied. The following types of derivatives were used:

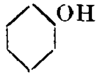
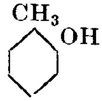
Benzene C_6H_6  ; Cyclohexane C_6H_{12}  ;

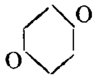
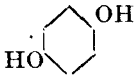
Hexane C_6H_{14} 

Aliphatic Alcohols: Methyl Alcohol $CH_3.OH$ to Amyl Alcohol $C_5H_{11}.OH$.

Ether $(C_2H_5)_2O$; Acetone $(CH_3)_2CO$; Chloroform $CHCl_3$; Formaldehyde $H.CHO$.

Benzene C_6H_6  ; Toluene $C_6H_5.CH_3$  .

Phenol $C_6H_5.OH$  ; Cresol $C_6H_4.CH_3.OH$  .

Quinone $C_6H_4O_2$  ; Hydroquinone $C_6H_4(OH)_2$  .

Pyridine C_5H_5N  , and certain inorganic substances.

The general results are as follows: Nearly all the antiseptics cause increases in bacterial numbers after treatment as Hiltner[1] has already pointed out, and they also mostly produce an initial increase in the amount of ammonia. Here, however, the uniformity ceases and closer examination shows that two very distinct classes of antiseptics exist:

(1) those which are completely volatile and disappear entirely from the soil once their work is done,

(2) those which remain in the soil for a considerable period or else leave decomposition products and so exert a prolonged action on the bacterial flora and on the plant.

1. The easily volatile antiseptics are all similar in their action but differ as regards their potency. When a particular strength is reached all the usual partial sterilisation effects observed by Russell and Hutchinson show up together: an initial depression in the numbers of bacteria followed by a large sustained rise, the killing of all protozoa, except a few flagellates, the killing of nitrifying organisms, and a small initial production of ammonia; followed later by a large increase in bacterial numbers and in the rate of production of ammonia. The methods used for the examination of the soils did not disclose any substantial difference in result with any higher dose once partial sterilisation had occurred. On the other hand the weakest doses have no effect on the numbers of bacteria occurring in the soil, nor on the rate of production of ammonia and nitrate, and, as far as can be judged from the ordinary one per cent. hay infusion culture method, no appreciable action on the protozoa. This absence of any effect on nitrate production of weak antiseptics, which are nevertheless powerful solvents (*e.g.* acetone), is strong evidence against the view expressed by Greig-Smith that the action of volatile antiseptics in increasing fertility is chiefly by the removal of "agricere." Typical results for chloroform

[1] *Arbeiten der Biolog. Abteilung f. Land u. Forstwirtschaft*, 1903, Bd. 3, Heft 5.

are shown in the curves of Fig. 1. The various amounts of the antiseptics required for effective action are given below:

	Effective dose, grams per kilo	Per cent. by weight of dry soil
Benzene	below M/50	below 0·15
Toluene	M/100	0·09
Cyclohexane .	M/50	0·17
Pentane	M/10[1]	0·7
Hexane	M/50[1]	0·17
Heptane	M/100[1]	0·1
Chloroform ..	below M/50	below 0·24
Ether	below M/5	below 1·5
Acetone	M	5·8

[1] At these strengths there are only indications of normal partial sterilisation, but increase of the dose to M has no further action.

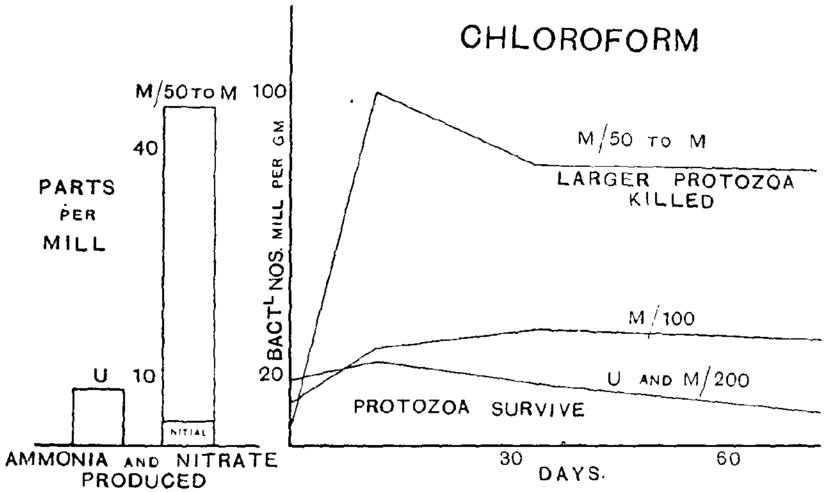


Fig. 1. Effect of chloroform on bacterial numbers, on protozoa, and on ammonia and nitrate production in soils.

It was noticed that all soils treated with a dose of volatile antiseptic in excess of that required for effective normal partial sterilisation moistened up badly after they had been dried in the spreading out. The water ran about on the surface of the soil and did not readily soak into the particles. This was most marked in the case of chloroform.

2. The group of less easily volatile substances includes not only the solid antiseptics which are effective although not readily removable even in the weakest doses, but also most of the alcohols, as by the time

that partial sterilisation phenomena show up the dose used is so high that traces of the alcohol remain in the soil after it has been spread out for evaporation to take place. Apart from these alcohols the members of this group all produce a permanent depression in bacterial numbers with the highest doses. The more potent such as quinone and hydroquinone show an initial depression in numbers of bacteria developing on gelatine plates even with the weakest dose used (approx. 0·05 per cent.), while the less potent show no initial effects with such strengths excepting the liberation of a small amount of ammonia. With all the antiseptics the dose which is sufficient to produce the full initial depression in numbers also kills the larger protozoa and checks the action of nitrifying organisms. The traces of substance left behind in the soil exert a very distinct action on the bacterial flora. The usual result is to produce at varying periods after treatment an enormous rise in the number of certain special organisms. This is seen not only in cases where the substance is known to be attachable by certain bacteria, *e.g.* alcohol and phenol[1], but in others such as quinone, where such action was not expected. The new flora is not the normal soil flora, although with the very mildly acting antiseptics the old flora may persist in approximately its original complexity but certainly in no greater numbers. The predominant part of the new flora is very much simpler even than that remaining after treatment with the easily volatile but potent antiseptics. The colonies are all very slowly growing and closer examination shows the presence of only two or three species of bacteria. Determinations of the nitrogen present in the soil as ammonia and nitrate show that the new flora is not an ammonia-producing one. When the abnormally high numbers fall off the condition finally attained depends on the intensity of the initial action: phenol and cresol in weak doses leave a flora which is similar in character to that arising after normal partial sterilisation and does produce a certain small amount of ammonia more than the untreated. None of these non-volatile antiseptics, however, leads to such a marked increase in the amount of ammonia and nitrate present after an incubation period as do the volatile antiseptics. This may be accounted for in part by our series of doses not happening to include the optimum for this result. It is also possible that the more active proteolytic forms in the soil are extinguished even by the very weak doses *or* that the antiseptics may have some action on the proteins of the soil rendering them less easily attackable by soil bacteria.

[1] Cp. Fowler, Ardern and Lockett, *Proc. Roy. Soc.* 1910, 83. B., 149.

Formaldehyde, which at the higher doses suppresses all life in the soil and gives results quite characteristic of this class, also helps to bridge the gap between the non-volatile and the volatile antiseptics. In very small doses it is remarkably similar in its action to the weak volatile antiseptics—the open chain hydrocarbons. They give an initial depression in bacterial numbers and a very distinct increase in the initial ammonia content but nitrifying organisms are not killed nor are the larger protozoa entirely suppressed. There is no distinct and permanent increase in the numbers of bacteria present although one would have expected this to be associated with the large increase in production of nitrate which undoubtedly occurs and which is of the same order as the increase in production of ammonia in the true partially sterilised soils. The action appears to be an intensification of that occurring after mere spreading out of the soil in a thin layer for 24 hours so that it dries down on the average to about five per cent. water before it is immediately remoistened. In this case there is a similar increased rate of production of nitrate over that in the soil which has been kept in a moist state after being bottled fairly fresh from the field, although there is no noticeable effect on the numbers of bacteria. The decreased action is indicated by the absence of such marked initial effects[1].

The curves (Fig. 2) obtained for the numbers of bacteria present after the treatment of the soil with non-volatile antiseptics are remarkably similar to those obtained by Hutchinson and MacLennan[2] with Woburn soil treated with various doses of quicklime. They attribute the large temporary rises to certain types of bacteria feeding on stores of food which before were unavailable but have been made accessible by the action of the caustic lime on the organic matter of the soil. In our case these high rises of numbers which are not sustained are also attributed to a "feeding effect" and range from the comparatively simple case of pyridine to the more complex one of hydroquinone.

Pyridine in doses up to 0·5 per cent. has no appreciable depressing effect on the flora prevailing in the soil before treatment and it seems probable from our experiments and from further investigations which we are carrying out, that the very large numbers (3500 million in one

[1] The numbers of bacteria are probably depressed in the untreated soil immediately after it is spread out, but the treated soils suffer an initial depression before they are spread out for volatilisation of the chemical. The cultural methods used did not demonstrate any action on the protozoa arising from the spreading out.

[2] Hutchinson, H B. and MacLennan, K., *Journ. Agr. Sci.* 1914, VI. p. 302.

case—a record in our laboratory with any soil) which arise after treatment from the development of two or three species of bacteria are due to these particular forms feeding directly on the pyridine itself and carrying out the decomposition process completely as far as the nitrogen is concerned to ammonia and nitrate.

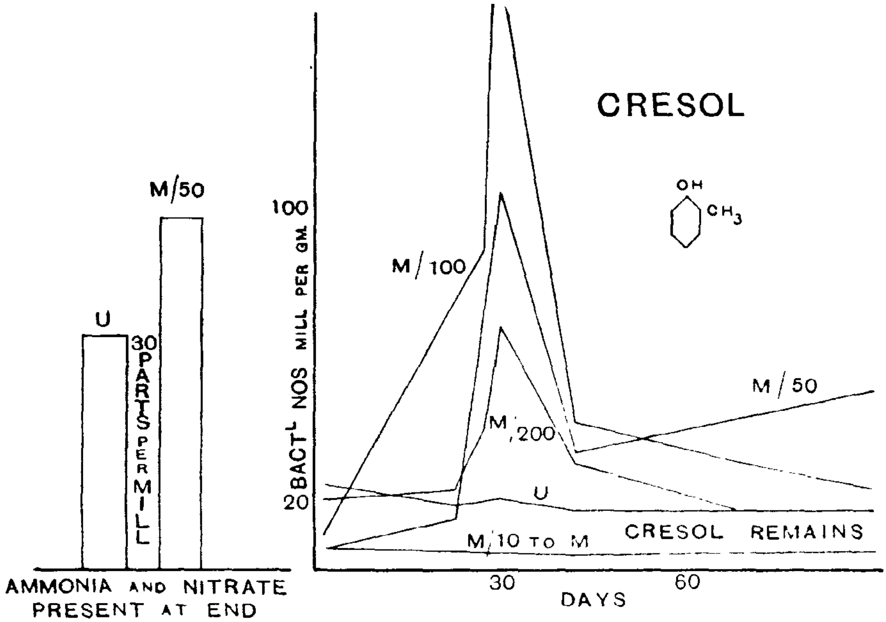


Fig. 2. Effect of cresol on bacterial numbers, and on ammonia and nitrate production in soils.

In the case of the other antiseptics the temporary rises are possibly due to the combined effect of special species of bacteria feeding on the antiseptic itself, on oxidation or condensation products of the antiseptic, or on substances which are not present in the normal untreated soil but are liberated from the organic matter of the soil by chemical action. Whatever may be the cause of these huge numbers of bacteria, as we have already pointed out they do not lead to any production of ammonia or nitrate.

Experiments with inorganic substances usually led to the result that either before or very soon after the partial sterilisation dose was reached the chemical action was so marked that it interfered seriously with the methods used for the examination of the soils.

The production of a small quantity of ammonia (about 4–8 parts per million of nitrogen calculated on the dry soil) immediately after

treatment is quite a constant feature throughout these experiments, and is the more remarkable in that it is produced by such chemically inert substances as toluene, etc. The following is a list of the substances and doses requisite to show this initial liberation of ammonia:

	Doses		Doses
Benzene	M/100 to M	Heptane	M/200 to M
Toluene	M/200 to M	Chloroform	M/100 to M
Phenol	M/200 to M/50	Ether	M/10 to M
Cresol	M/200 to M/50	Acetone	M/10 to M
Cyclohexane	M/100 to M/5	Formaldehyde	M/200 to M/50
Pentane	M/10 to M	Calcium Sulphide	M/10 to M[1]
Hexane	M/100 to M	Sodium Fluoride	M/5 and M[1]

[1] In these cases dark coloured water extracts indicated some chemical change.

The initial action of pyridine is unknown as in our determinations of ammonia immediately after treatment the pyridine distilled over and was alkaline to methyl orange—the indicator then used.

The production of a small quantity of ammonia initially has *not* been noted with the following substances:

Quinone,	Hydroquinone,
Sulphur dioxide,	The aliphatic alcohols.

The narrowness of the limits within which the good effects of partial sterilisation may be able to show up with a non-volatile substance is illustrated by the action of phenol. Russell and Petherbridge had observed the increased growth of tomato plants in "sick" soil treated with 0·25 per cent. of carbolic acid and at the same time obtained evidence from the character of growth of the plants that such a dose was excessive. Similar results were obtained by us with 0·2 per cent. in 1913, and higher doses have since been found in the laboratory permanently to depress bacterial action, yet experiments conducted in bottles with weak doses up to 0·1 per cent. phenol showed it to have none of the usual partial sterilisation effects[1]. This small range of effective action is in direct contrast to the wide range observed with volatile antiseptics where any strength ranging from 0·1 to 10 per cent. produces the same result. At the same time we must not lose sight of the possibility that a weak dose of such a substance as phenol might show to advantage in heavy manured glasshouse soil well stocked with disease organisms by killing these alone even if it produced no true partial sterilisation of the soil.

[1] The differences between the various soils used in these experiments was probably not sufficient to give rise to any serious narrowing of these limits.

It is highly desirable that an efficient chemical antiseptic should be found. Russell and his collaborators have already shown the application of partial sterilisation methods to be a cure for a wide range of troubles, but the cost of steam heating and baking which has been reduced to a minimum by the combined ideas and experience of many of the most competent nurserymen, prevents the economic application of these methods over a more expansive field. The price of a chemical is likely to be considerably more affected by an increased demand, but again there are certain restrictions. The use of volatile antiseptics involves practical difficulties—ineffective penetration of the lumps of soil by the vapour, impossibility of mixing the substance thoroughly with a sufficient depth of soil, cost of carriage and danger of working with inflammable materials—which are now well known but have not yet been overcome. A solid, however, would be most convenient in practice: it could be easily carted, weighed up and distributed and could be thoroughly mixed with the soil during the course of the ordinary routine work. Unfortunately these experiments have not brought to light a suitable new antiseptic, but they have emphasised and explained the value of phenol and cresol and have emphasised although they have not fully explained the value of formaldehyde.

It is worthy of note that in our experiments with tomato plants in pots during 1913 the development of fibrous root after treating the soil with 0·2 per cent. phenol was quite as great as in the steamed soil. This was not associated with any marked increase in fruit production, but all the plants had been fed equally with a mixture containing sulphate of ammonia, sulphate of potash and superphosphate. The increased development of fibrous root over that found in untreated soil has not been noticed in commercial nurseries where large quantities of carbolic are used although usually at a weaker dose and the result in our series of pot experiments may be quite accidental.

Experimental.

The experiments were conducted chiefly with soil from the Laboratory Allotment, but in the autumn of 1913, after being fallow throughout the summer, this soil became so rich in nitrates that it was thought advisable to use fresh soil from Sawpit Field: this was in good condition, but of low nitrate content, having carried a crop of wheat after potatoes. The analyses of typical samples from these fields are given below:

Percentage Calculated on the air dried soil.

Soil	Moisture	Loss on ignition	Nitrogen	Total P_2O_5	Avail. P_2O_5	Total K_2O	Avail. K_2O	Carbonates as $CaCO_3$
Allotment	2·3	12·0	·33	·35	·13	·31	·07	·31
Sawpit			·20	·15	·028	·38	·02	·6

The general method adopted was that used by Russell and Hutchinson in their experiments. The soil was brought straight in from the field, spread out and roughly picked over to remove worms and stones, sieved in a comparatively moist state through a 3 mm. sieve, thoroughly mixed and weighed up into bottles in 800 gram lots. The soils were then treated with the desired chemical either by mixing the antiseptic with water and sprinkling the soil so as to ensure thorough distribution, or by carefully mixing a solid with the soil and shaking up at short intervals; the bottles were tightly corked for two days, and afterwards plugged with sterile cotton wool. The wide range of doses adopted allowed of better opportunity of striking the most efficient strength, and in order to obtain as good a comparison as possible between the antiseptics used we thought it desirable—instead of adding fractions of 1 %—to treat the soil with definite fractions of a molecular weight in grams (M) of the chemical. The doses used were M/200, M/100, M/50, M/10, M/5, and where the price was not prohibitive M/1 per kilo of dry soil, *i.e.* varying approximately from 0·03 to 5·0 per cent. by weight.

After treatment with all the easily volatile compounds and with some of the less volatile, the treated soils were spread out in a fairly thin layer for 20–24 hours to allow the antiseptic to disappear from the soil as much as possible. In each set two untreated bottles were put up so that we might form some more definite idea of the differences to be expected between duplicate soils: when any of the soils were spread out one of the untreated was spread with them while the other was left in the moist state in the bottle. After volatilisation all the soils were moistened up to a uniform moisture content, varying in the different series from 15 % to 20 % water.

The method used for the determination of the ammonia present in the soils was that described by Russell[1], while the Zn-Cu couple reduction method was used for the determination of nitrate, the ammonia produced being estimated by distillation into excess of standard acid.

[1] *Journ. Agric. Sci.* 1910, III. p. 233.

The Readily Volatile Antiseptics.

Benzene is an excellent example of an easily volatile chemically inert antiseptic and shows the perfectly normal partial sterilisation results. The numbers of bacteria in the soil as determined by the gelatine plate method are initially depressed by a sufficient dose of the antiseptic to about one-third of those present in the untreated soil, but when the benzene has been removed by volatilisation and the soil moistened up the numbers rise rapidly to four or five times those in the untreated and remain constant at that level. This sustained rise in the numbers of bacteria occurs at the same point as the killing of the protozoa indicated by repeated cultures, and also at the death point of nitrifying organisms, while associated with it is a marked increase in the rate of production of ammonia, 31 parts of nitrogen as ammonia being produced in the effectively treated soils in the same time as 12 parts of nitrogen as nitrate are produced in the untreated. The obscure but definite initial production of about four parts per million of ammonia is a very common feature of the treated soils.

I. *BENZENE. Allotment Soil.* 16 % *Moisture.*

Grams of Benzene added per kilo of *dry* soil	Bacteria present. Millions per gram of soil			Ammonia and nitrate present, parts per mill. of dry soil as N		Effect on Protozoa
	At start	After 25 days	After 47 days	At start	After 26 days	
gms.						
Untreated[1] = 0	19	20	20	24·5	36·5	C A M
M/200 = 0·39	20	29	17·5	26	38·5	C (A) M
M/100 = 0·78	10	30	23	30	41·5	C A M
M/50 = 1·56	8	78	85	30	60·5	(M)
M/10 = 7·80	7	90	91	29·5	59·5	None present
M/5 = 15·6	6	90	92	27	63·5	(M)
M = 78·0	6·5	74	75	30	61	None present

Throughout the paper, in this column,

C indicates the presence of Ciliated Protozoa.

A ,, ,, ,, Amoebae.

M ,, ,, ,, Flagellated Protozoa.

( ) indicates that members of this particular group were not always found in the various cultures.

[1] As in all other cases where any of the soils were 'spread out' the figures given here are those obtained with the untreated soil which was 'spread out.'

The weakest dose—M/200—has practically no effect either on bacterial numbers or on the protozoa. A large proportion of the species of bacteria survive even the highest dose, so that the plates obtained after an incubation period of the soil show very mixed types of colonies.

It will be noticed from Table I that the partial sterilisation point occurs somewhere between the M/100 and M/50 dose.

Toluene, a Benzene ring with an added Methyl group, gives rise to no new features in the results obtained from these experiments, but shows all the normal partial sterilisation effects. It differs from Benzene only in being slightly more potent—the sustained rise in bacterial numbers etc. occurring with the M/100 dose.

II. *TOLUENE. Allotment Soil.* 16 % *Moisture.*

Grams of Toluene added per kilo of dry soil	Bacteria present. Millions per gram of dry soil			Ammonia and nitrate present, parts per mill. of dry soil		Effect on Protozoa
	At start	After 34 days	After 51 days	At start	After 30 days	
gms.						
Untreated = 0	22	16	18	24	43	C A M
M/200 = 0·46	16	22·5	18·5	26·5	50	C M
M/100 = 0·92	8·5	76	92	28·5	56	M
M/50 = 1·84	7	87	94	29	63	(M)
M/10 = 9·2	8	95	87	29·5	65	None present
M/5 = 18·4	8	77	90	29·5	66	„ „
M = 92·0	7	90	86	30	66	„ „

Cyclohexane and Hexane. Comparing completely reduced Benzene or Cyclohexane and the corresponding open chain compound, Hexane, with Benzene itself we find that Cyclohexane shows the normal partial sterilisation effect but is less potent. It begins to act at about M/50, but protozoa are not so completely killed off and the bacterial numbers do not rise so high[1]. Nitrification is active at all doses and from M/50 considerably more nitrate is produced than in the untreated soil.

Hexane, however, is relatively impotent and gives only indications of partial sterilisation. There is an initial production of ammonia and

[1] Difficulty was experienced at first in obtaining reliable counts of the numbers of bacteria prevailing after treatment owing to the hot weather and to the presence of a large proportion of actively liquefying organisms. The counts given above, however, obtained later in the incubation period, represent accurately the relative numbers prevailing in the various soils.

an initial depression in bacterial numbers, but a small rise above the untreated soil is not maintained, there being practically no difference between the later counts. Protozoa are slightly affected, but repeated cultures show that many species still survive. The difference between the amount of nitrate present in the treated soils and in the 'spread out' untreated soil at intervals subsequent to treatment is very little more than is accounted for by the initial production of about 7·5 parts per million of ammonia, and thus there is no definite indication of any actual increase in the rate of production of ammonia in the treated soils.

III. *CYCLOHEXANE. Allotment Soil.* 19 % *Moisture.*

Grams of Cyclohexane added per kilo of dry soil	Bacteria present. Millions per gram of dry soil			Ammonia and nitrate present, parts per million of dry soil			Effect on Protozoa
	At start	After 37 days	After 92 days	Initial Ammonia	At start	After 103 days	
gms.							
Untreated = 0	14	14	12	3	27	52·5	C A M
M/200 = 0·42	16	12	—	4·5	25·5	55	C A M
M/100 = 0·84	9	12	10·5	10	34	57·5	(C A) M
M/50 = 1·68	10·5	30	41	10	34	70·5	(A) M
M/10 = 8·40	6	35	34	10·5	31·5	76	M
M/5 = 16·8	11	31·5	43	10	31	77·5	(A) M

IV. *HEXANE. Allotment Soil.* 19 % *Moisture.*

Grams of Hexane added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per million of dry soil			Effect on Protozoa
	At start	After 19 days	After 57 days	After 103 days	Initial Ammonia	At start	After 119 days	
gms.								
Untreated = 0	23	18	17	17	4·5	26	56·5	C A M
M/200 = 0·43	14·5	19	17	16	4·5	27	58	C A M
M/100 = 0·86	22·5	18	16	17·5	8	28	59	(C A) M
M/50 = 1·72	11	22·5	18	22	12·5	33·5	65	(C) A M
M/10 = 8·6	8	33·5	25	25	11	30	68	C (A) M
M/5 = 17·2	6·5	42·5	25	20	12	33	65	A M
M = 86·0	8·5	—	25	28·5	—	—	67·5	(A) M

Heptane and Pentane. These open chain hydrocarbons also give indications of partial sterilisation, but the poisoning property appears to be too mild to produce the full effect. They resemble the more

potent volatile compounds such as Toluene in that increase in bulk of antiseptic to even twentyfold does not seem to have any further effect once the amount has been sufficient to show any partially sterilising action. Heptane begins to act at M/100 and here has a deleterious effect on the larger protozoa, but there is not even a well-marked initial depressing effect on the numbers of bacteria while they never multiply appreciably above the untreated level. There is, however, a small initial production of ammonia and an increase in the amount of nitrate produced with all the doses used.

Pentane is less potent and has no effect either on protozoa or on the accumulation of nitrate until the M/10 dose is reached, but at this strength in addition to an initial production of ammonia there is a reduction in the initial number of bacteria to about half those occurring in the untreated soil.

V. *HEPTANE. Sawpit Soil.* 15 % *Moisture.*

Grams of Heptane added per kilo of dry soil	Bacteria present. Millions per gram of dry soil			Ammonia and nitrate present, parts per million of dry soil			Effect on Protozoa
	At start	After 24 days	After 63 days	Initial Ammonia	At start	After 71 days	
gms.							
Untreated = 0	20	20	13	3·5	22·5	49·5	C A M
M/200 = 0·5	23·5	15	15	5·5	25·5	62·5	C A M
M/100 = 1·0	19	22	15·5	7·5	28	62·5	(C) A M
M/50 = 2·0	19	24	16·5	7	26	64	(C) A M
M/10 = 10·0	17	16·5	16·5	9·5	29	66	A M
M/5 = 20·0	15	20·5	17·5	9·5	28·5	61	(C) A M

VI. *PENTANE. Sawpit Soil.* 15 % *Moisture.*

Grams of Pentane added per kilo of dry soil	Bacteria present. Millions per gram of dry soil			Ammonia and nitrate present, parts per million of dry soil			Effect on Protozoa
	At start	After 30 days	After 53 days	Initial Ammonia	At start	After 60 days	
gms.							
Untreated = 0	15	14·5	12	3·5	22·5	36	C A M
M/200 and M/100	16	13·5	12	3	23·5	34·5	C A M
M/50 = 1·44	17·5	11	13	3·5	26	39	C A M
M/10 = 7·2	8	10	14	9·5	30	53·5	(C A) M
M/5 = 14·4	8·5	17·5	20	9	28	55	(C) A M
M = 72·0	9	21	24	—	—	58	(A) M

Formaldehyde. The soils treated with Formaldehyde were spread out for 24 hours in a thin layer similarly to the soils treated with the easily volatile substances in order to allow the antiseptic to pass off as much as possible, but Schiff's reagent showed that with the higher doses some remained in the soil throughout the experiment and kept the soil in a practically sterile condition. Protozoa were seriously affected at

VII. *FORMALDEHYDE. Allotment Soil.* 17 % *Moisture.*

Gms. of Formaldehyde added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Amm. and nit. present, parts per mill. of dry soil		Effect on Protozoa
	At start	After 22 days	After 57 days	After 90 days	At start	After 37 days	
gms.							
Untreated = 0	17	28	17	12	28·5	57·5	C A M
M/200 = 0·15	13	25	7	7·5	33·5	71·5	(C A) M
M/100 = 0·30	5	25	26	18	25·5	63·5	M
M/50 = 0·60	5	25	5	9	18	60	None present
M/10 to M	0·1	0·5	1	3	16	22	,, ,,

Reinfected set. Sawpit Soil. 14·5 % *Moisture.*

Treatment	Bacteria present. Millions per gram of dry soil				Amm. and nit. present, parts per million of dry soil			Effect on Protozoa
	At start	After 37 days	After 125 days	After 170 days	At start	After 61 days	After 174 days	
Untreated	17	14	14	12·5	28	36	50·5	C A M
M/100		16·5	14	22		49·5	71	
M/100 Reinf.[1] at 2 days	3·5	17	13	15	27	49	64·5	(C A) M
M/100 ,, 29 & 65 ,,		14	16	20		52	67	
M/200		11	13·5	13·5		47	55·5	
M/200 Reinf. at 2 days	5·5	12·5	12	16	26·5	52	58·5	C A M
M/200 ,, 29 & 65 ,,		9·5	13·5	20		49	63·5	
Allotment Soil	At start	After 16 days	After 102 days	After 155 days	At start		After 156 days	
Untreated Not 'spread'	10	11·5	12	12	31		36	C A M
Untreated 'Spread out'	14·5	8	12	10·5	29·5		44·5	C A M
M/125		7·5	11	6			54	
M/125 Reinf. at 2 days	8	7·5	13	7	24		55	(C A) M
M/125 ,, 29 & 114 ,,		—	14	8			53	

[1] This consisted in reinfection with the flora of the untreated by addition of 0·5 per cent. of the untreated soil.

M/200 and entirely suppressed at M/50, while a distinct initial depression in bacterial numbers occurred at M/100. These weak doses are remarkably similar in their results to those obtained with the larger doses of the open chain hydrocarbons we have just studied. They all showed a small initial production of ammonia. Inhibition of nitrification was rather erratic, but considerably more nitrate was produced in the M/200 soil in 37 days than in the untreated, although there was no corresponding sustained rise in the numbers of bacteria growing on gelatine plates. Reinfected sets with weak doses of Formaldehyde confirmed the results without throwing much light on the cause. It is possible that the Formaldehyde soils contain many bacteria which grow extremely slowly, if at all, on meat extract gelatine.

Consistently good results have been obtained in pot experiments from the treatment of soils with Formaldehyde for the growth of tomatoes, the crop obtained being both earlier and greater in bulk.

The Alcohols.

The alcohols are less active than might be supposed and by the time the partially sterilising dose is reached they mostly come within the class of incompletely volatile antiseptics, disturbing factors thus being introduced.

Methyl Alcohol with both Sawpit and Allotment Soil is inactive until the M dose is reached and even then nitrification is active while the larger protozoa are not completely suppressed. Distinctly more

VIII. *METHYL ALCOHOL.*

Grams of Methyl Alcohol added per kilo of dry soil	Bacteria present. Millions per gram of dry soil			Ammonia and nitrate present, parts per mill. of dry soil		Effect on Protozoa
Allotment Soil 17·5 % moisture	At start	After 12 days	After 55 days	At start	After 32 days	
Untreated = 0 gm.	16	15	14	30·5	48	C A M
M/200 to M/5	16	13	11	29	46	C A M
M = 32·0 gms.	5	28·5	38·5	31	70	(C A) M
Sawpit Soil 16 % moisture	At start	After 12 days	After 61 days	At start	After 74 days	
Untreated = 0 gm.	8·5	11	18	14	31·5	C A M
M/200 to M/5	6·5	12	13	14	33	C A M
M = 32·0 gms.	7	46	46	16	60·5	(C A) M

nitrate is produced while the numbers of bacteria rise to three times the numbers in the untreated soil; with Allotment Soil there is a definite initial depression in bacteria.

Ethyl Alcohol is also not active till the M dose, but at that strength it inhibits nitrification and also appears to have rather more suppressing effect on the protozoa than Methyl Alcohol. At the same time traces of alcohol seem to remain behind in the soil after it has been spread

IX. *ETHYL ALCOHOL. Allotment Soil.* 17·5 % *Moisture.*

Grams of Ethyl Alcohol added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Nitrate present, parts per million of dry soil		Ammonia and nitrate present, parts per million of dry soil		Effect on Protozoa
	At start	After 11 days	After 25 days	After 57 days	At start	After 30 days	At start	After 30 days	
gms.									
Untreated = 0	13	18	16	13	33	40·5	35	43·5	C A M
M/200 = 0·23	17	23	19	16	30	36·5	32·5	40·5	C A M
M/100 = 0·46	21·5	50	43	20·5	23	34	25	38	C A M
M/50 = 0·92	20	76	42	35	23	19·5	25	23·5	C A M
M/10 = 4·6	14	20	23	8·5	33	31	35·5	33·5	C A M
M/5 = 9·2	10·5	35	30·5	18·5	32·5	34	37	38	C A M
M = 46·0	7	505	310	92	33·5	16	36	39	(A) M

Sawpit Soil. 16 % *Moisture.*

Grams of Ethyl Alcohol added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per mill. of dry soil		Effect on Protozoa
	At start	After 15 days	After 26 days	After 66 days	At start	After 74 days	
gms.							
Untreated = 0	7	13	20	10	12	28	C A M
M/200 to M/10	7	13·5	20	12	9	24	C A M
M/5 = 9·2	7	13	24	15	11·5	38·5	C A M
M = 46·0	3·5	58	107	74	12	73	M

out for 20 hours for volatilisation to take place and apparently serving as food lead to abnormally high numbers of bacteria. Counts in the treated Sawpit Soil show the presence of 100 millions of bacteria, while in Allotment Soil the numbers rise to a maximum of 500 millions: this high level is not maintained and there is a subsequent

fall to 90 millions per gram. In the case of both soils more ammonia is formed in the effectively treated samples than in the untreated, but with Allotment Soil several irregularities occur as regards the nitrate. With the M dose, which gives rise to huge numbers of bacteria, the amount of nitrate falls off from 33·5 parts present initially to 16 parts per million present after 30 days, and a similar effect is seen with the M/50 dose where there is a rise in the numbers of bacteria to 76 millions per gram and a slight falling off in the nitrate as compared with 20 million bacteria in the untreated and M/200 samples and an increase in nitrate content of seven parts per million in 30 days.

Iso- and Normal Propyl Alcohols.

Iso-Propyl alcohol was tried only up to a strength of M/5 per kilo of dry soil and this gave merely indications of partially sterilising

X. *ISO-PROPYL ALCOHOL. Allotment Soil.* 16·5 % *Moisture.*

Grams of Alcohol added per kilo of dry soil	Bacteria present. Millions per gram of dry soil			Ammonia and nitrate present, parts per million of dry soil		Effect on Protozoa
	At start	After 24 days	After 92 days	At start	After 106 days	
gms.						
Untreated = 0	14	13	9	29	39·5	C A M
M/200 to M/50	13	14	13	27	40	C A M
M/10 = 6·0	14	28	12	28	37·5	C A M
M/5 = 12·0	13·5	28·5	28	30·5	49	C A M

X*a*. *NORMAL PROPYL ALCOHOL. Allotment Soil.* 16·5 % *Moisture.*

Grams of Alcohol added per kilo of dry soil	Bacteria present. Millions per gram of dry soil					Ammonia and nitrate present, parts per mill. of dry soil		Effect on Protozoa
	At start	After 21 days	After 32 days	After 84 days	After 118 days	At start	After 126 days	
gms.								
Untreated = 0	17	7·5	14	8	15·5	26	43	C A M
M/200 to M/50	21	26	40	12	17·5	23	36	C A M
M/10 = 6·0	12·5	46	49	20	26	27	41·5	C A M
M/5 = 12·0	11	87	206	76·5	85	28	62	(A) M
M = 60·0	5	200	416	113	103	—	53	M

action; the numbers of bacteria were permanently slightly higher than in the untreated and there was a small but definite increase in the amount of nitrate produced.

Normal Propyl alcohol again shows huge temporary counts, giving in one case 465 millions of bacteria per gram at 29 days, but there is also a distinct suppression of protozoa with both the M/5 and M doses. While the former leads to a very distinct increase in the amount of nitrate produced, the latter stops nitrification and yields an increased amount of ammonia. Thus it appears that as we rise in the series the alcohols become more and more potent as partially sterilising agents.

Amyl Alcohol. No extensive series of experiments has been carried out with Amyl Alcohol, but it remains in the soil and apparently supplies bacterial food even with as low a dose as 0·1 per cent. or approximately M/100. Using Allotment and Vine "Sick" Soil counts were obtained as high as 180 and 290 millions per gram, as compared with 30 and 50 respectively in the Untreated soils, while the numbers were still up to half these values at the end of 50 days, but in neither case was quite as much nitrate formed as in the control soils. In other experiments with both Sawpit and Allotment Soils counts as high as 130 millions compared with 12 in the Untreated have been obtained after treatment with 0·1 per cent. amyl alcohol.

Other Open Chain derivatives.

Perfectly normal partial sterilisation results have also been obtained by the use of the volatile compounds—chloroform, ether and acetone.

Chloroform. The M/200 dose gives results exactly similar to those obtained with the untreated 'spread' soil, but the full antiseptic action shows up between the M/100 and M/50 strengths. The rise in numbers of bacteria set in rapidly after treatment but reliable counts were somewhat difficult to obtain owing to the fact that 25 per cent. of the colonies coming up after treatment were rapid liquefyers probably *B. liq. fluor.* However, the results show certainly that the high level of 80–100 million bacteria per gram was maintained in all the partially sterilised soils (Fig. 1).

Ether acts between M/10 and M/5, *i.e.* at a strength of a little more than 7·4 grams per kilo of dry soil. M/200, M/100 and M/50 doses all give substantially the same results as the untreated.

XI. *CHLOROFORM. Sawpit Soil.* 15 % *Moisture.*

Grams of Chloroform added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per million of dry soil			Effect on Protozoa
	At start	After 12 days	After 34 days	After 72 days	Initial Ammonia	At start	After 42 days	
gms.								
Untreated = 0	18	25	18	11	3	25	29·5	C A M
M/200 = 0·59	14	28	24	11·5	4·5	24	33·5	C A M
M/100 = 1·19	11	27	35·5	31	6·5	21·5[1]	44	C A M
M/50 = 2·38	4	81	68	187 ?	6·5	21·5[1]	64	(A) M
M/10 = 11·93	6	89·5	59	64	6·5	21·5[1]	69	M
M/5 = 23·86	4	115	51	97	6·5	21[1]	65	M
M = 119·3	7	90	63	76	6	19[1]	66	M

[1] I attribute the low values obtained for the initial nitrate content to the soil wetting imperfectly immediately after treatment so that the nitrate was not completely washed out. It was noticed that all soils treated with a dose of volatile antiseptic in excess of that required for effective partial sterilisation moistened badly after they had been dried in the spreading out. This was most marked in the case of chloroform. Samples of soil for the determination of the nitrate present at the start are dried in the oven at 55° C. before lixiviation.

XII. *ETHER. Sawpit Soil.* 15 % *Moisture.*

Grams of Ether added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per million of dry soil			Effect on Protozoa
	At start	After 14 days	After 46 days	After 61 days	Initial Amm.	At start	After 33 days	
gms.								
Untreated 0	21	17	15	—	3	25	39	C A M
M/200 to M/50	22	15	15	—	4	24	44	C A M
M/10 = 7·4	15	41·5	36·5	—	8·5	28	50	C A M
M/5 = 14·8	4	96	66·5	102	8·5	28	81	M
M = 74·0	4·5	81	89	57	8·5	26·5	71·5	M
ACETONE Sawpit Soil	At start	After 12 days	After 56 days			At start	After 68 days	
Untreated = 0	20	25	22			25	32	C A M
M/200 to M/5	28	23	21			24	31	C A M
M = 58·0 gms.	6	88	60			24·5	68	M

Acetone does not act until the M dose is reached but then shows all the usual partial sterilisation phenomena. Soil treated with strengths of antiseptic ranging from M/200 to M/5 shows no difference from the untreated.

Phenol derivatives.

The non-volatile antiseptics, as has been already pointed out, are under the disadvantage that excess remains behind in the soil after treatment and exercises a prolonged influence on the microbiological changes going on in the soil. We have seen that the volatile hydrocarbons, Pentane, Heptane, etc., differ from the more active volatile compounds such as Toluene in that they are unable to bring about complete partial sterilisation however great the dose; on the other hand the non-volatile compounds we have tried up to the present seem to err in the direction of being too drastic in their action on the microflora of the soil even in the weakest doses, unless the dose be so small that it has no material effect on any of the soil organisms.

So far in our bottle experiments we have been unable to obtain a strongly developing mixed flora in the treated soil by subsequently reinfecting with the flora of the untreated soil although there would seem to be more scope for its development than in a reinfected toluened soil whose flora always remains comparatively complex. The evidence is by no means conclusive but the possibility remains that the antiseptic may have some permanent action on the proteins similar to the well-known hardening action of formaldehyde on gelatine.

Phenol in the weaker doses shows high rises in the numbers of bacteria similar to the other hydroxy compounds—the aliphatic alcohols—but in the stronger doses is very potent in its action. On Allotment soil with as low a dose as 0·01 per cent. the bacterial numbers showed no initial depression but rose very rapidly to 130 millions per gram, and even up to 0·1 per cent. the phenol had no action on the protozoa; the production of nitrate was neither accelerated not hindered, about 18 parts per million being formed in all the soils during the period of the experiment, while the final counts showed that the bacterial numbers in all the soils had fallen to the untreated level of 20 millions per gram. In a complete set with the same soil no attempt was made to eliminate excess of the antiseptic after treatment and the soil was kept moist in bulk in the bottles, receiving only a very occasional shake. Doses from M/10 to M kept the soil life in an inactive condition for the 75 days of the experiment. Phenol persisted in the soil, protozoa were entirely killed and the numbers of bacteria were kept down at 5 millions per gram. Large temporary rises in bacterial numbers were found in all the weak doses and appeared to be associated with a disappearance of some of the phenol. The highest count recorded was 548 millions in

the M/100 soil after a lapse of 16 days, while the flora was very simple judging from the few prevailing types of colonies on the gelatine plates. Larger protozoa were suppressed between the M/100 and M/50 doses, and there was a small initial liberation of ammonia, but no appreciable amount of ammonia or nitrate accumulated in any of the soils. In a reinfected set with Sawpit soil, however, nitrification was stopped by the M/50 dose and distinctly more ammonia accumulated than was formed in the untreated soil.

XIII. *PHENOL. Allotment Soil.* 16·5 % *Moisture.*

Grams of Phenol added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per mill. of dry soil			Effect on Protozoa
	At start	After 16 days	After 31 days	After 74 days	Initial Amm.	At start	After 74 days	
gms.								
Untreated = 0	23	28	16	15	4·5	28·5	30·5	C A M
M/200 = 0·47	17	101	59	18·5	6·5	26·5	32	C A M
M/100 = 0·94	12	548	96	28·5	8	29·5	34·5	(C A) M
M/50 = 1·88	7·5	13·5	114	41	6·5	25·5	26·5	M
M/10 to M	5	4	3	4	4·5	24	23	None pres.

Reinfected set. Sawpit Soil. 15 % *Moisture.*

Treatment	Bacteria present. Millions per gram of dry soil					Ammonia and nitrate present, parts per mill. of dry soil			Effect on Protozoa
	At start	After 13 days	After 39 days	After 56 days	After 97 days	Initial Amm.	At start	After 97 days	
Untreated	24	23	22	18	14	3	26	27	C A M
M/100	14	212	30	21·5	39	6	25	31	C A M
M/100 Reinf. at 2 days		217	40	26	34			33·5	C A M
M/100 „ 21 „		–	26	27·5	48			32	C A M
M/50	6	7	24	19·5	23·5	6·5	23	41	M
M/50 Reinf. at 2 days		5	17·5	20·5	28			46·5	M
M/50 „ 21 „		–	59	47·5	57·5			45	M

The apparently greater numbers of bacteria present in the M/50 soil reinfected after an interval with the untreated flora, than in the other M/50 soils led to no greater formation of ammonia. Brown streptothrix were fairly common on the plates obtained from this as on

those from the untreated and M/100 soils, while they were practically absent from the other M/50 plates.

The higher doses as with other non-volatile antiseptics seem to lead to a low value for the initial nitrate content of the soil, but the cause of this has not yet been determined. It may be noted that the extract obtained on washing out the nitrate is of a bright yellow colour.

The use of phenol in strengths varying from 0·1 to 0·25 per cent. on the weight of the soil has given consistently good results with tomato plants in pots, the early growth being much better and the crop of fruit earlier than in the untreated. The final yield of fruit has usually been very little better than in the untreated, as all the plants have been fed equally during the later stages of growth. In one series a remarkably increased development of fibrous root comparable with that in the heated soil was observed.

Cresol is similar in action to phenol but is slightly less abnormal in its results. It does not have quite such a depressing effect on ammonia production in the soil and the bacteria which tolerate it do not multiply to such huge numbers, the highest count recorded being 164 millions. The main set showed that protozoa are practically suppressed at M/50 and this dose also resulted in a very distinct accumulation of ammonia. Higher doses remained in the soil and suppressed all bacterial action. A reinfected set showed differences in the amount of ammonia and nitrate produced, but these were not correlated with the numbers of bacteria present; they rather appeared to depend on whether or not the active ammonia producers succeeded in establishing themselves after reinfection. Simple non-ammonia producing floras seem capable

XIV. *CRESOL. Allotment Soil.* 16·5 % *Moisture.*

Grams of Cresol added per kilo of dry soil	Bacteria present. Millions per gram of dry soil					Ammonia and nitrate present, parts per mill. of dry soil			Effect on Protozoa
	At start	After 22 days	After 30 days	After 66 days	After 112 days	Initial Amm.	At start	After 119 days	
gms.									
Untreated 0	23·5	15	19	17·5	17	4·5	32	34	C A M
M/200 = 0·54	19	21	56·5	22	36·5	7·5	29·5	40	C A M
M/100 = 1·08	11	86[1]	164	30	22·5	6·5	27	41	A M
M/50 = 2·16	6	15	106	33	52	8	29·5	48·5	M
M/10 to M	4	2·5	3	4	6	4	25·5	28·5	None present

[1] 27 days.

Reinfected Set. Allotment Soil. 19 % *Moisture.*

Treatment	Bacteria present. Millions per gram of dry soil: At start	After 11 days	After 45 days	After 80 days	After 145 days	Ammonia present, parts per million of dry soil: At start	After 156 days	Ammonia and nitrate present, parts per million of dry soil: At start	After 156 days	Effect on Protozoa
Untreated	22	21·5	17	14·5	13	2	4	26	34	C A M
M/100		83	51	32·5	43		2		41	
M/100 Reinf. at 2 days	10·5	181	57	43	30	5	3·5	23	40·5	C A M
M/100 „ 24 „		—	51	56	42		18·5		54·5	
M/50		4	45·5	22	19·5		25·5		42·5	
M/50 Reinf. at 2 days	8	4	36	31	36	5	48·5	20·5	75	M
M/50 „ 24 „		—	132	51	70		35		43·5	

of reaching higher numbers than the more complex ones actively producing ammonia. Meta- and para-cresol at the M/75 dose were found to give rise also to temporary high numbers of bacteria and act similarly to ortho-cresol, but neither appeared to be quite so effective.

Quinone is very drastic in its action on protozoa and also on bacteria even down to the M/200 dose, but in no case have we obtained the slightest evidence of more ammonia and nitrate being produced than in the untreated soil, and so far as our experiments go it seems to be too powerful an antiseptic to be of use as a partially sterilising agent. Although the initial action is so potent and lasting yet certain bacteria are able to withstand it: the M/10 dose kept down the numbers of bacteria below one million for at least 24 days, yet when some of the quinone had disappeared one or two species were able to thrive and multiplied rapidly to 160 millions per gram. With the weaker doses the rise in numbers takes place earlier in the incubation period. That these extraordinary numbers of bacteria are not associated with any increased production of nitrate indicates that the simple flora is probably feeding on the quinone or on some decomposition product. The low results obtained in the nitrate determinations, especially the initial, with the high doses, and all depressing effects are more marked with quinone than with phenol and cresol (Fig. 3).

In a reinfected set with the M/100 and M/200 doses all except two gave higher counts than the untreated, but there was very little difference in the final values for total ammonia and nitrate. It was noticed that brown streptothrix showed up strongly on all the plates

from the soils giving high counts, while it was practically absent from the others. In a repeat reinfected set with Allotment soil and M/125 dose some very active liquefyers were present in the quinone treated soils and made counting difficult, but the numbers certainly did not reach a high level and the flora was very simple even after reinfection.

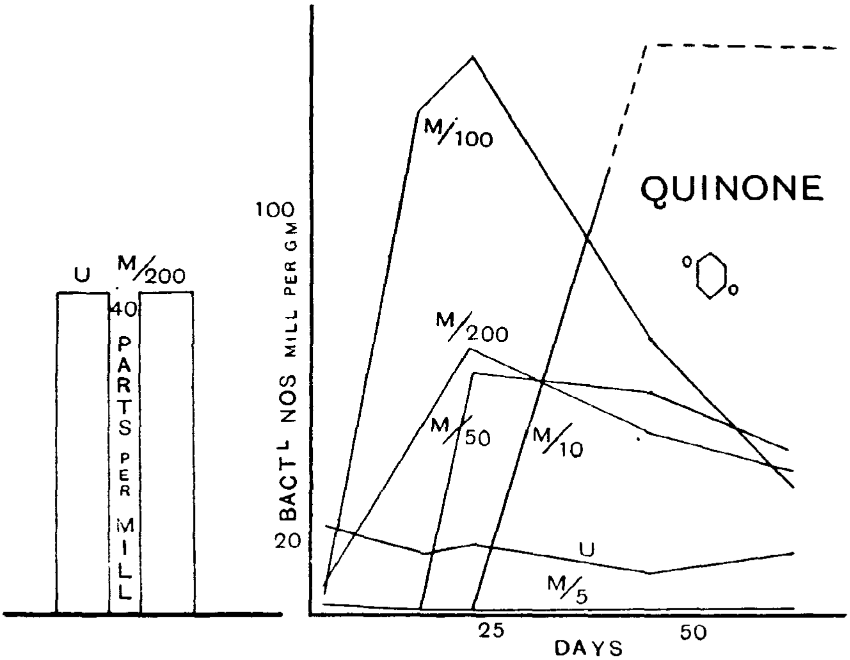


Fig. 3. Effect of quinone on bacterial numbers and on ammonia and nitrate production in soils.

XV. *QUINONE. Allotment Soil.* 19·5 % *Moisture.*

Grams of Quinone added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per mill. dry soil		Effect on Protozoa
	At start	After 16 days	After 50 days	After 71 days	At start	After 71 days	
gms.							
Untreated = 0	24	16	10	16	37	42	C A M
M/200 = 0·54	7	47	46·5	36	32·5	40	C (A) M
M/100 = 1·08	5	126	68	33	32	31·5	M
M/50 = 2·16	2·5	1	55	41·5	30	32	M
M/10 = 10·8	2·5	0·1	157	159	19	27	None pres.
M/5 = 21·6	1	0·1	0·1	4	19	19	,, ,,

Reinfected Sets.

Treatment	Bacteria present. Millions per gram of dry soil					Ammonia and nitrate present, parts per mill. dry soil		Effect on Protozoa
	At start	After 11 days	After 35 days	After 84 days	After 235 days	At start	After 94 days	
Sawpit Soil. 14·5 % *moisture*								
Untreated	21	19	16	21	15	24	25	C A M
M/100		2	7	10	—		25·5	M
M/100 Reinf. at 2 days	1	11	69	65	42	18·5	19·5	(C) M
M/100 ,, 26 ,,		—	8·5	13·5	10		20·5	M
M/200..............		26	62	73	—		28·5	C A M
M/200 Reinf. at 2 days	3	27·5	92	74	49	24	28	(C A) M
M/200 ,, 26 ,,		—	40	61	42		31	(C A) M
	At start	After 32 days	After 91 days	After 105 days	After 162 days	At start	After 174 days	
Allotment Soil								
Untreated	10	12	13	8	3·5	24·5	33·5	C A M
M/125..............		9	7	10	3		29·5	
M/125 Reinf. at 2 days	3·5	31	5	9	6·5	22	26·5	M
M/125 ,, 33 ,,		51	6	7	7		33	

Hydroquinone is very similar in behaviour to quinone. It is a little more drastic in its initial action on bacteria, but does not appear to have quite such a depressing effect on nitrate production and does not lead to such high occasional numbers of bacteria.

Pyridine—a nitrogen ring compound and therefore possessing a certain manurial value—produces some very remarkable results. A preliminary small set of experiments showed that even with as low a dose as 0·01 per cent. it is impossible to volatilise all the Pyridine from the

XVI. *HYDROQUINONE. Allotment Soil.* 17·5 % *Moisture.*

Grams of Hydroquinone added per kilo of dry soil	Bacteria present Millions per gram of dry soil			Ammonia and nitrate present, parts per mill. dry soil		Effect on Protozoa
	At start	After 24 days	After 79 days	At start	After 79 days	
gms.						
Untreated 0	20	16	15	29	35	C A M
M/200 0·55	4	55	30	26	44	C A M
M/100 1·1	1	20	24	24	35	(C A) M
M/50 = 2·2	1	61	15	22	30	M
M/10 to M	0·1	0·1	0·3	22	21	None present

Reinfected Sets.

Treatment	Bacteria present. Millions per gram of dry soil					Ammonia and nitrate present, parts per mill. dry soil		Effect on Protozoa
	At start	After 10 days	After 58 days	After 80 days	After 119 days	At start	After 119 days	
Sawpit Soil. 15 % moisture								
Untreated	18	26	22	18	17	22	33	C A M
M/100..............		1	1	6	20		22·5	
M/100 Reinf. at 2 days	1	2	2	10·5	61[1]	20	26	
M/100 ,, 23 ,,		—	8	14	16		26·5	
M/200..............		12	14·5	12	48[1]		34·5	M
M/200 Reinf. at 2 days	4·5	16	14	25·5	20	22·5	36	
M/200 ,, 23 ,,		—	14	27·5	29·5		36·5	
Allotment Soil	At start	After 32 days	After 105 days	After 162 days		At start	After 113 days	
Untreated	10	12	8	3·5		24·5	30	C A M
M/125..............		11	6·5	3			24·5	
M/125 Reinf. at 2 days	3	6	5·5	1		22	27	M
M/125 ,, 33 ,,		36·5	5·5	5			24	

[1] High count largely due to a very slowly growing yellow colony.

soil by spreading out for 24 hours, so that in order to control conditions as far as possible and to ensure greater uniformity between the bottles in the same series the treated soils were not spread out in our main sets.

From these and other experiments it appears that pyridine affords a magnificent diet for bacteria and provides the simple case of two or three species feeding directly on the substance itself or on its decomposition products. The increasing doses show successive temporary high rises in bacterial numbers culminating in the presence of 3,500 million bacteria per gram of soil with the M/10 dose after a period of 86 days, and these high numbers seem to be associated with a disappearance of some of the pyridine. Similar very high counts have been obtained with both Sawpit and Allotment Soil in other experiments. Protozoa are not suppressed until the M/5 dose which remains in the soil and keeps down the numbers of bacteria over a period of 80 days. The ammonia and nitrate determinations are complicated by the nitrogen contained in the pyridine itself, *e.g.* the M/10 dose corresponds to an addition of 1,400 parts per million of nitrogen to the soil, and in the estimation of ammonia in the soils the greater part of the pyridine distils over, remains in the distillate and gives an alkaline reaction with methyl orange. This effect can be overcome to a certain extent by the

XVII. *PYRIDINE.* *Allotment Soil.* 18·5 % *Moisture.*

Grams of Pyridine added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Present in parts per mill. of dry soil: Nitrate		Ammonia and nitrate	Effect on Protozoa
	At start	After 16 days	After 30 days	After 86 days	At start	After 82 days	After 82 days[1]	
gms.								
Untreated = 0	29	32	26	13	30	36	36	C A M
M/200 = 0·39	24·5	138	78	19.	24·5	75	75	C A M
M/100 = 0·79	30·5	315	173	28·5	28·5	115	115	C A M
M/50 = 1·58	32·5	123	942	35	28	193·5	193·5	C A M
M/10 = 7·9	22	26	16·5	3500	25	64	432	(C A) M
M/5 = 15·8	12	16·5	8	7	34·5	27	46	(M)
M/1 = 79·0	7	12	5	3	39·5	27	34	None present

[1] Phenolphthalein was used as indicator in the ammonia estimation. Methyl orange was used in the initial determinations and the results were consequently vitiated by the pyridine present in the soil. The figures obtained were M/200 = 44; M/100 = 93; M/50 = 161; M/10 = 921; M/5 = 1130, and M = 1800 parts per million.

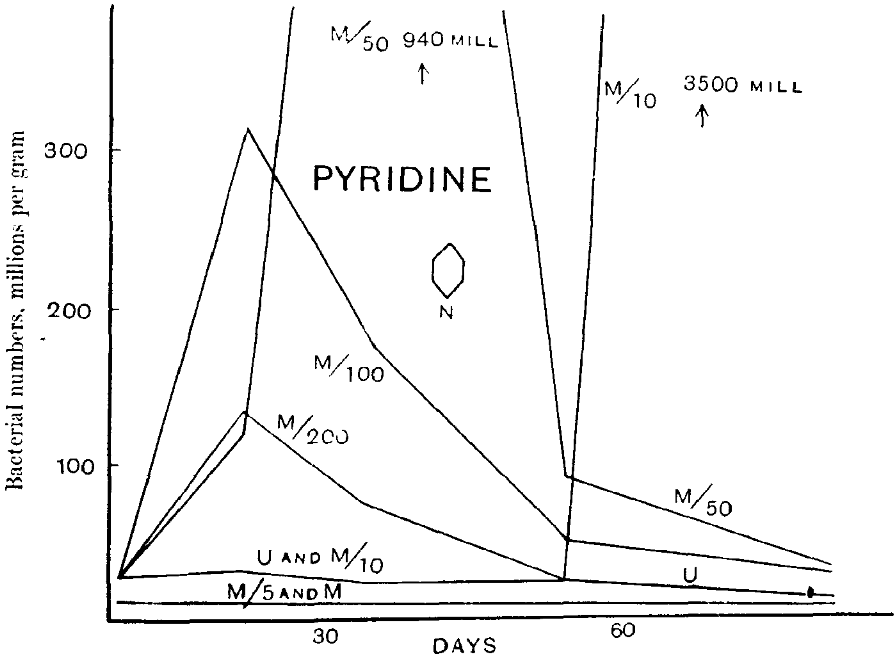


Fig. 4. Effect of pyridine on bacterial numbers in soils.

use of phenolphthalein as indicator, this being neutral to pyridine but alkaline to ammonia. The results also indicate that the pyridine is converted in the soil to nitrate through ammonia, there being present finally 368 parts of ammonia in the M/10 soil and in the M/50 soil 193 parts of nitrate or of a soluble compound reducible by the zinc-copper couple to ammonia. These amounts are far greater than any obtained in normally partially sterilised soils. In the soil treated with the M/5 dose which was sufficient to prevent the bacteria multiplying only the normal amount of ammonia and nitrate is found (Fig. 4).

With pyridine therefore neither the high bacterial numbers nor the large quantities of nitrate found subsequent to treatment are due to true partial sterilisation, but are characteristics of the particular substance used. Our other indicators—the suppression of protozoa and the initial depression in bacterial numbers—show that true partial sterilisation does not occur until after the M/10 dose.

Inorganic Antiseptics.

Calcium Sulphide was tried as it is the active agent in gas lime known to have sterilising value. In the laboratory it had already shown uncertain results as a partially sterilising agent.

Russell and Hutchinson had found that a small dose of calcium sulphide, although it had no appreciable effect on the amount of nitrate produced, in two cases led to an initial depression in the numbers of bacteria, while with one soil at the end of 26 days, 42·5 million bacteria were present in the soil treated with Calcium Sulphide as compared with 8·5 in the untreated[1]. Pot experiments showed 0·25 % Calcium Sulphide to have a bad effect on the first crop, but a beneficial effect on the second crop following treatment.

	Dry weight of crop.	
	1st Barley	2nd Buckwheat
Untreated	2·4 gms.	1·5 gms.
0·25 % CaS	0·5 ,,	3·3 ,,

Russell and Petherbridge[2] had also found it to have a good effect on the growth of chrysanthemums, particularly on the size of the blooms.

Preliminary bottle experiments with 0·1 % showed that it was without much effect on a vine "sick" soil, but on the Allotment soil

[1] Russell, E. J. and Hutchinson, H. B., *Journ. Agric. Sci.* 1913, v. 173.
[2] Russell, E. J. and Petherbridge, F. R., *Journ. Agric. Sci.* 1913, v. 248.

29 parts of nitrate were found compared with 13 in the untreated although there was no apparent effect on bacterial numbers.

The main series showed that with Allotment soil bacteria begin to be depressed initially at the M/50 dose, but protozoa are not seriously affected till the M/5 dose (or 1·2 % by weight on the moist soil) is reached. The M dose keeps the soil in an almost sterile condition and has a considerable chemical action on the soil, 23·5 parts of ammonia being found immediately after treatment. Although the action does not show up so prominently in the M/10 and M/5 soils, yet no doubt the chemical action lightens the work of the ammonia producing bacteria.

XVIII. *CALCIUM SULPHIDE. Allotment Soil.* 18 % *Moisture.*

Grams of Calcium Sulphide added per kilo of dry soil	Bacteria present. Millions per gram of dry soil			Nitrate present (Parts per million of dry soil)		Ammonia and nitrate present (Parts per million of dry soil)		Effect on Protozoa
	At start	After 19 days	After 61 days	At start	After 68 days	At start	After 68 days	
gms.								
Untreated = 0	40	36	24	21	32	24	38·5	C A M
M/200 and M/100	47	43	33	22	38·5	25·5	44	C A M
M/50 = 1·44	28	44	23	21·5	44	24·5	49	C A M
M/10 = 7·2	21	38	62	(7·5)	59·5	17·5	69·5	C A M
M/5 = 14·4	10	43	79	(15)	(4·5)	24·5	64·5	(C A) M
M/1 = 72·0	2	3	1·5	(19·5)	(11·4)	43	45	None present

While the unchanged calcium sulphide is still present in quantity in the soil it leads to a low value being obtained for the nitrate[1], but later in the incubation period this effect disappears and 59·5 parts per million of nitrogen as nitrate were found after 68 days in the M/10 soil. Considerably more ammonia was also produced in the M/5 soil than in the untreated, while both showed finally distinctly higher numbers of bacteria. It is difficult to differentiate between the chemical and

[1] This has been shown to be due probably to the calcium sulphide being washed out of the soil in the extraction and then interfering with the reduction of the zinc-copper couple. 1·2 gms. of calcium sulphide (corresponding to the amount present in 200 gms. of the soil treated with the M/10 dose) were shaken thoroughly with water, allowed to stand and filtered off. To the filtrate was then added 20 c.c. of a standard nitrate solution and the boiling down with magnesia, acidifying with acetic acid and reduction carried out in the usual way. 20 c.c. of the standard nitrate solution were found to contain 4 m.gms. of N, but the solution treated with calcium sulphide yielded only 0·7 m.gm. of N as ammonia on distillation.

partially sterilising actions, but neither in these experiments produced any substantial benefit until a strength of antiseptic was reached equal to between 0·12 and 0·6 per cent. by weight on the moist soil.

Sulphur. Several investigators record increased crop results from the application of small quantities of sulphur in the field, but it is to be noted that in practically all the experiments observations were made on root crops which are well known to give unreliable results unless taken over large areas. It has been suggested by some that the result may be due to the partial sterilisation of the soil by the sulphur[1].

In experiments by Pfeiffer and Blanck[2] the plots receiving sulphur compared unfavourably with the control plots both as regards the yield obtained after an application of sulphur and the utilisation of the nitrogen of the soil. The authors considered that the alleged favourable results of other experimenters were due in some measure to insufficient provision against experimental error.

Again in the Woburn pot experiments[3] small dressings of sulphur applied to soil in which mustard, rape and clover were grown had no influence from the beginning of the experiment nor on the final weights of crop. W. Jonicaud[4], however, using tomatoes in pots as the test plant and treating the soil with 0·2 per cent. flowers of sulphur came to the conclusion that even in the presence of a complete manure sulphur stimulates the growth of the plant. The increases recorded in the numbers of bacteria, viz. from 12·5 millions to 23·5 in the unmanured and from 11 to 16 in the fully manured, are quite small compared with the increase obtained in our normally partially sterilised soils and we should not lay much stress on such differences unless they remained constant over several determinations.

In our experiments using both Allotment and Vine "Sick" soil 0·1 per cent. of sulphur had practically no effect either on bacterial numbers or on the production of nitrate.

Russell and Hutchinson[5] found that 0·25 % flowers of sulphur on an arable soil suppressed nitrification and ammonification for 30 days and kept the numbers of bacteria down to half those prevailing in the untreated. In our experiments with allotment soil 0·5 per cent. of

[1] *e.g.* Chancrin and Desriot. *J. Agri. Prat.* n. ser. 21 (1911), No. 14, pp. 427–429. Abst. in *E. S. R.* xxv p. 519.

[2] *Landw. Versuchs. Stationen*, Bd. 83, Heft 5 and 6. p. 359.

[3] Report of the Woburn Expt. Stn. on Pot Culture Expts., *Journ. Roy. Agric. Soc.* 1913.

[4] Abst. in *New Zealand Journ. of Agri.* VIII. No. 4. p. 412.

[5] This *Journal*, 1913, v. 173.

sulphur had no effect either on the bacterial numbers or on protozoa, while there was a falling off in the amount of nitrate present during 120 days.

Thus there is practically no evidence that sulphur acts as a partially sterilising agent.

XIX. 0·1 *per cent. Flowers of Sulphur.*

Allotment Soil	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per mill. of dry soil	
	At start	After 13 days	After 55 days	After 70 days	At start	After 70 days
Untreated	36	23	30	27	24·5	37
0·1 % Sulphur	31	35	36·5	33	23·5	38
0·1 % Toluene	12	104	115	200	29	57
Vine "Sick" Soil	At start	After 9 days		After 50 days	At start	After 50 days
Untreated	50	69		57·5	136	150
0·1 % Sulphur	50	59		50	136	136
0·1 % Toluene	11	82		92	146	159

0·5 *per cent. Flowers of Sulphur. Allotment Soil.* 19 % *Moisture.*

	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per mill. of dry soil		Effect on Protozoa
	At start	After 19 days	After 57 days	After 103 days	At start	After 120 days	
Untreated	23	20	19·5	19·5	25	41	C A M
0·5 % Sulphur....	16	20	16	10·5	28	13	C A M

Sulphur Dioxide has no appreciable partially sterilising action until it produces a distinct chemical reaction. A saturated solution was prepared, diluted as required and watered on to the various soils, the M/10 dose which was the strongest used producing distinct efferves-cence. Smaller doses had no effect on bacterial numbers and led to only a small increase in nitrate. The M/10 dose depressed the numbers

of bacteria and the protozoa initially, but subsequently all developed strongly. All types of protozoa were represented and the bacteria multiplied to 100 millions per gram. There was a distinct increase in the amount of nitrate produced.

XX. *SULPHUR DIOXIDE. Sawpit Soil.* 15 % *Moisture.*

Grams of Sulphur Dioxide added per kilo of dry soil	Bacteria present. Millions per gram of dry soil				Ammonia and nitrate present, parts per mill. of dry soil		Effect on Protozoa
	At start	After 17 days	After 29 days	After 85 days	At start	After 91 days	
gms.							
Untreated = 0	23	23·5	19	13·5	25	34·5	C A M
M/500 and M/200	21	20	23	16	25·5	38	C A M
M/100 = 0·64	19	18	18	13	23·5	41·5	C A M
M/50 = 1·28	16·5	22	21	15·5	28	45·5	C A M
M/10 = 6·4	7·5	101	70	42	18·5	56	C A M

Potassium Permanganate. The effect of a single dose (M/50) of this well-known antiseptic was tried on allotment soil. This strength had no appreciable effect on protozoa, but the initial amount of ammonia in the soil rose from 5 to 10 parts per million of nitrogen, while the amount of nitrate as indicated by the reduction method was increased initially very considerably from 24 to 41 parts. The number of bacteria present after treatment was about twice as high as in the untreated soil, being increased from 20 to 40 millions per gram, but no increase in the amount of ammonia and nitrate took place over a period of 79 days. Thus the M/50 dose of potassium permanganate does not appear to have any partially sterilising action.

Sodium Fluoride has distinct antiseptic properties, but when added to soil in effective quantities it has a considerable chemical action. Protozoa and bacteria are not affected till the M/5 dose is reached, but M/50 produced a distinct increase in the initial value for nitrate, and the marked chemical action is illustrated by the fact that the extract obtained after washing out the nitrate is practically black in colour from both the M/5 and M soils. The amount of nitrate present increased in these soils during the experiment, but with such extracts small differences in the manipulation probably have a very considerable effect on the amount of ammonia obtained from the final distillation,

and it is impossible to say how far the nitrate production is influenced by the increased numbers of bacteria alone.

Sodium Chloride. For purposes of comparison we also put up a set with common salt. The M dose suppressed protozoa and kept the numbers of bacteria slightly below the untreated numbers. It gave a dark brown extract in the nitrate determination, but did not lead to high values as did the black extracts from the soils treated with sodium fluoride. None of the doses gave rise to increased numbers of bacteria in the soils, but nitrification was stopped at the M/5 dose, and this also led to an increased production of ammonia. Normal partial sterilisation cannot be said to have occurred at any strength used.

XXI. *SODIUM FLUORIDE and SODIUM CHLORIDE.*
Allotment Soil. 18 % *Moisture.*

Grams of *Sodium Fluoride* added per kilo of dry soil	Bacteria present. Millions per gram of dry soil			Ammonia and nitrate present, parts per mill. of dry soil		Effect on Protozoa
	At start	After 39 days	After 76 days	At start	After 89 days	
gms.						
Untreated = 0	36	28	22	34	40	C A M
M/200 and M/100	31	35	21	34·5	41·5	C A M
M/50 = 0·84	42	28	22·5	42	48	C A M
M/10 = 4·2	36	25	18	45·5	74	C A M
M/5 = 8·4	29	70	62	60	146	C (A) M
M = 42·0	15	23	82	78	177	None present
Sodium Chloride	At start	After 39 days	After 77 days	At start	After 84 days	
Untreated = 0	25	22	21	33	40	C A M
M/200 to M/50	30	24	26	35	38	C A M
M/10 = 5·85	40	18	26	32	50	C A M
M/5 = 11·7	64	25	25	32	56	C A M
M = 58·5	20	14	17	31	35·5	M.

Summary and Conclusions.

1. The characteristics of true partial sterilisation have been found to be common to a large number of antiseptics and are

(*a*) An initial decrease in the numbers of bacteria followed by a large sustained rise.

(*b*) The killing of protozoa and nitrifying organisms. In no case have we observed all the usual partial sterilisation phenomena without

the death of the larger protozoa which occur abundantly in cultures made from our soils.

(*c*) An initial increase in ammonia content followed by a considerable increase in the rate of production of ammonia, and consequently in productiveness.

(*d*) No increase in dose causes any change in the results obtained once true partial sterilisation has set in with any particular chemical.

2. True partial sterilisation has been obtained only with the easily volatile or removable antiseptics. It is essential for its detection that all the tests be combined: neither bacterial nor chemical examination of the soils alone is sufficient and even the combined results from a new substance must be compared with the results from a well-known antiseptic such as toluene.

3. Substances not completely removable from the soil have some lasting influence on the flora. With the weaker doses two or three special species of bacteria characteristic of the chemical used multiply temporarily to an enormous extent; but the organisms do not produce ammonia, consequently there is no gain in ammonia and nitrate as the result of their action. The higher doses permanently suppress all microbiological action in the soil.

4. It appears to be a general rule that a simple flora can attain extraordinarily high numbers while a complex flora, such as prevails after normal partial sterilisation, does not attain to higher numbers than the comparatively low level of about five times those in the untreated.

5. It is possible to trace a certain relationship between the action of all the substances used. The intensity of the effects shades off gradually from that of the powerful non-volatile antiseptics through cresol (M/50 dose) and formaldehyde to the more and less potent volatile antiseptics respectively, till finally the action of merely spreading out the soil in a thin layer is reached.

6. Volatile antiseptics are undoubtedly effective in increasing the productive capacity of a soil under laboratory and pot culture house conditions, but are unsuitable for application on the larger scale. An efficient solid substance would be very convenient in use and probably much cheaper than methods of partial sterilisation by heat. Unfortunately our experiments have not justified the setting up of any extensive series of pot trials and have not revealed any suitable new non-volatile substance. They have, however, emphasised the value

and explained the action of phenol and cresol and have emphasised, although they have not explained, the action of formaldehyde.

7. We are hoping to obtain more information about the special species of bacteria which are able to withstand unusual doses of the potent poisons and subsequently to multiply rapidly and produce practically pure cultures in the soils.

In conclusion the writer wishes to express his great indebtedness to Dr E. J. Russell at whose suggestion the work was undertaken and whose advice and assistance throughout have been invaluable.

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NOTE ON THE INCREASED NITRATE CONTENT OF A SOIL SUBJECTED TO TEMPORARY DRYING IN THE LABORATORY.

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An interesting factor governing the nitrate content of a soil has been brought out in the course of the preceding work on the treatment of soil with volatile compounds.

In the main sets of experiments two series of untreated bottles were put up. The initial determinations were made while the antiseptics were still present, so that the two initial untreated soils were true duplicates and gave results agreeing within the usual experimental error. When the antiseptic had been allowed to act for two days the treated soils were spread out in a thin layer for the chemical to evaporate as far as possible, and one series (*B*) of the untreated soils were spread out together with them while the other series (*A*) of untreated were kept moist in the bottle. After the soils had been left for 20 to 24 hours they were rebottled and the whole set were moistened up as soon as possible to a uniform water content. The soil as bottled had undergone the usual sieving and mixing process, was in a comparatively fresh condition and contained from 10 to 18 per cent. of moisture. When they were spread out the soils dried down on the average to 95 per cent. dry matter and in spite of the immediate remoistening determinations made after an incubation period showed that these 'dried' or 'spread out' soils (*B*) contained distinctly more nitrate than the samples of the same soil which had been kept permanently moist (*A*). Counts by the gelatine plate method of the number of bacteria in the soils were made at intervals varying from 10 days to 5 months, but in none of the 20 sets was there any significant difference between the numbers of bacteria occurring in the soils. The average[1] of 68 individual

[1] No difference was observed between the results obtained from Allotment soil and those from Sawpit field soil, both of which were used in these experiments and the results have been grouped together for this average.

counts showed that 16 millions of bacteria were present in series *A* compared with 15 millions in series *B*, while the average initial numbers in the 20 sets were 17 millions in *A* and 16 in *B*. The difference of one million bacteria per gram is therefore probably no more than the experimental error of the method, but in any case less bacteria rather than more were present in the soils that had been spread out than in the same soils kept moist throughout.

Average results for 20 *sets.*

	Numbers of bacteria present, millions per gram of dry soil		Nitrogen as Ammonia and Nitrate, parts per million of dry soil	
	Initial	During incubation period	Present initially	Formed during incubation period
Series *A*, kept moist ..	17	16	24·5	9·5
Series *B*, 'spread out' and remoistened..	16	15	24·5	17·5

In no set was more nitrate produced in series *A* than in series *B*. The average difference in favour of series *B* throughout the 20 sets was 8 parts per million of nitrogen as nitrate calculated on the dry soil, nearly twice as much nitrate being produced during the incubation period as in the soils kept permanently moist. The probable error of this average calculated from the usual formula is ± 2·4. The increase in the amount of nitrate produced being more than three times the probable error the odds are considerable that in any soil put up under our conditions more nitrate would be found after an incubation period in the sample spread out for 20—24 hours and remoistened than in the sample kept moist.

In considering this result we were handicapped by the fact that no determinations of ammonia and nitrate were made immediately after the soils were spread out and the initial values obtained for the sets of soils treated with volatile antiseptics are not true initial values as regards this special side issue. It was possible that the soils might have absorbed from the atmosphere an amount of ammonia sufficient to account for the final difference observed. To test this point a set of bottles was put up in which the determinations were made immediately after spreading out and again at an interval of 40 days. Some of the soils were spread on the gallery of the old Rothamsted Laboratory,

as most of the previous sets had been in a comparatively pure week end atmosphere, while other samples were spread out in the glasshouse. These dried down rather more than most of our other soils had done, the gallery spread soils containing 96 per cent. dry matter and the glasshouse spread soil 98 per cent., and a little more nitrate and ammonia was found initially than in the untreated permanently moist soil, but again nearly twice as much nitrate was formed during the incubation period in the spread soils as in the samples which had not been spread out.

Nitrogen as ammonia and nitrate, parts per million of dry soil.

	Immediately after spreading out	After lapse of 40 days	Increase
Untreated, moist soil	24	36	12
Spread on gallery and remoistened	26	46	20
Spread in glasshouse and remoistened	30	53·5	23·5

In a further set with Allotment soil containing originally 15 per cent. moisture, samples were spread out for intervals varying from 2 to 40 hours and the soil dried down correspondingly to from 87 to 96 per cent. dry matter. The numbers of bacteria were depressed immediately after spreading out from 10 millions in the untreated moist soil to four or five million in the soils spread out for 24 or 40 hours, but in all cases the numbers rose subsequently to about the same level. Initial determinations of ammonia and nitrate showed no marked difference between the soils, but after a period of 87 days the untreated samples and those spread out for periods up to 12 hours contained from 28 to 33 parts per million of nitrogen as ammonia and nitrate compared with 41 parts in the samples spread out for 24 and 40 hours.

Several sets were put up subsequently with soils spread out for 24 and 46 hours for the determination of ammonia and nitrate immediately after drying and these did not show any definite differences in the initial figures. Some samples of soil were also spread out on copper gauze over concentrated sulphuric acid in a closed atmosphere under a large bell jar, and these again failed to show any real initial difference from the untreated.

Thus the increased amount of nitrate found after an incubation period in soils which have been spread out for 24 hours and then remoistened over that in similar samples which have been kept moist

appears to be due not to absorption from the atmosphere but to the formation of more nitrate from the residues in the soil in spite of the fact that the numbers of bacteria are not increased.

Nitrogen as ammonia and nitrate immediately after spreading.

	Average of 6 separate determinations			Average of 3 separate determinations	
	Dry matter %	Parts per mill.		Dry matter %	Parts per mill.
Unspread	83·5	41·4*	Unspread	84	47·8
Spread 24 hours	96	41·3	Spread over sulphuric acid	98	47·3
„ 46 „	97	43·1			

* The average figures are rather higher than the usual initial values with this soil owing to most of the samples being taken from the fallow field late in the summer and also manure had been applied in the spring to that part of the Allotment from which some samples were removed.

Up to the present we have found no satisfactory explanation of this raising of the 'limit' of nitrate accumulation by spreading out the soil, but the results are of importance in that they show the necessity of spreading the untreated soil side by side with the treated in all work with volatile antiseptics. Neglect to observe this stage in the endeavour to treat all the soils exactly alike may lead to serious error, especially with the mild poisons whose action both on numbers of bacteria and on accumulation of nitrate is small. Weak doses of formaldehyde and the less potent volatile antiseptics—the open chain hydrocarbons—show a similar but much more intensified action. The initial effects on bacteria, protozoa and ammonia content are more marked with these treated soils than with the 'spread out' soils.

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THE EVAPORATION OF WATER FROM SOIL.

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Introduction.

The relation between the soil and the soil water is very complex, because it is the resultant effect of many variables. The moisture is subject to percolation and evaporation, the effects of which in the field are constantly varying, as they depend, among other things, on the amount of water present, the meteorological conditions and that important combination of vague factors known as "the state of the soil." Although the problem has received much attention it is far from being solved. The earlier investigators regarded the soil as a framework of particles covered with a continuous film of water. This simple hypothesis has the advantage that it is susceptible of mathematical treatment and allows a number of important physical deductions to be made. For instance, from considerations of surface tension it is possible to calculate the theoretical distribution of the enveloping water films over the surface and in the interstices of the soil grains, and, as a logical development to trace the movement of water from regions of high moisture content to places initially containing a small amount of water. This view has been developed in a number of interesting papers by Cameron and Gallagher[1], Briggs[2], Buckingham[3], Leather[4], and others.

But it has become increasingly evident that such a treatment of the problem does not completely explain all the observed facts, and that the connection between the soil and the soil water is of a closer nature

[1] U. S. Bureau of Soils, Bulletin 50.
[2] *Ibid.*, Bulletin 10.
[3] *Ibid.*, Bulletin 38.
[4] *Memoirs of Indian Agric. Dept. Pusa.* Chemical Series, Vol. I. No. 6.

than is indicated by the above hypothesis. The recognition of the existence of soil colloids has thrown fresh light on the subject and rendered further advance possible. One of the most promising methods of attack is to study the evaporation of the water from the soil under rigidly controlled conditions. This has the further advantage that as evaporation is essentially a surface phenomenon it yields useful evidence on the nature and effect of the soil colloids.

The experiments recorded in the present paper have yielded the significant result that evaporation of water from soil differs fundamentally from evaporation from sand or silt.

When a tray of moist sand is suspended over sulphuric acid (the method adopted in this paper) the rate of evaporation is largely determined by the rate of diffusion of the water vapour from the sand to the acid, and the observed results agree closely with those calculated from the laws of diffusion. But soil behaves entirely differently: something it contains influences the process of evaporation, so that the comparatively simple laws holding in the case of sand or silt do not apply. Experiments to discover which constituents of soil are responsible for this difference showed that the soluble "humus[1]" plays little part, evaporation being only very slightly affected by its removal from the soil. But ignition entirely alters the character of the evaporation, causing it to become precisely similar to the evaporation from sand or silt. Now ignition not only removes the organic matter but also destroys the colloidal properties of the soil. Evidence is adduced to show that the loss of organic matter is not *per se* the determining factor. The results show that the destruction of the colloidal nature of the soil causes the type of evaporation to change over from that given by soil, to that given by sand. The soil fraction termed "clay" thus becomes of especial interest, because it is this fraction which shows colloidal properties in the most marked degree. At present little can be said about the exact manner in which the colloid material is distributed in the soil. There is evidence, however, that its distribution is more or less general, and that it forms a coating over the surface of the soil grains.

The evaporation from soil has been examined in detail and two factors have here been distinguished and developed mathematically. One factor expresses the effect of surface on the rate of evaporation. The water initially present in the soil is of course distributed over the soil grains probably as part of the colloidal film mentioned above. As

[1] *I.e.* the material removed by 2% $NaOH$.

evaporation proceeds the thickness of the film decreases, and hence the available *surface* from which evaporation can take place also decreases. Now, evaporation is essentially a surface phenomenon, and, other things being equal, is the more rapid the larger the surface. Thus, in the present case, the rate of evaporation will constantly decrease, owing to the progressively diminishing surface.

The second, and more important factor, is an empirical expression, which expresses mainly the effect of the *vapour pressure* of the moist soil on the rate of evaporation. During the evaporation the moisture content of the soil is constantly decreasing and therefore the vapour pressure falls off. In other words the driving force producing vaporisation of the soil water becomes less and less, and thus the rate of evaporation decreases also. The complete explanation of this factor, which, as stated above, is at present empirical, demands a knowledge of the relation between vapour pressure and water content, and this relation is now being further investigated. It seems probable that it will be found to be directly connected with the colloidal properties of the soil.

Previous work of other investigators.

The work of Patten and Gallagher[1] may be taken as typical of previous investigations on this subject. The moist soil was held in weighing bottles placed over sulphuric acid of varying strengths in desiccators kept at a constant temperature. The bottles were removed at intervals and weighed. The result led to the unexpected conclusion that the rate of evaporation did not change between wide ranges of moisture content, being the same for such diverse substances as moist quartz flour and heavily manured cotton soils. A repetition of their experiments with Hoos field dunged soil, and moist fine sand (obtained by the usual mechanical analysis and then ignited to destroy organic matter) gave similar results. These are plotted in Fig. 1, and if the method gave a true measure of the evaporation their significance would be great. For the linear relation between percentage of water and time shown by these curves is the same as that given by a free water surface. This would mean that the soil water is present in the free state down to at least 4% and that the soil was behaving merely as a framework for the water films. The parallelism of the curves for widely differing soils would also indicate that the

[1] U. S. Bureau of Soils, Bulletin 51: "Absorption of gases and vapours by soils."

nature and structure of the soil have no influence on the rate of evaporation. But a critical examination of the method shows that the results given by it should not be regarded as quantitative. The thick layer

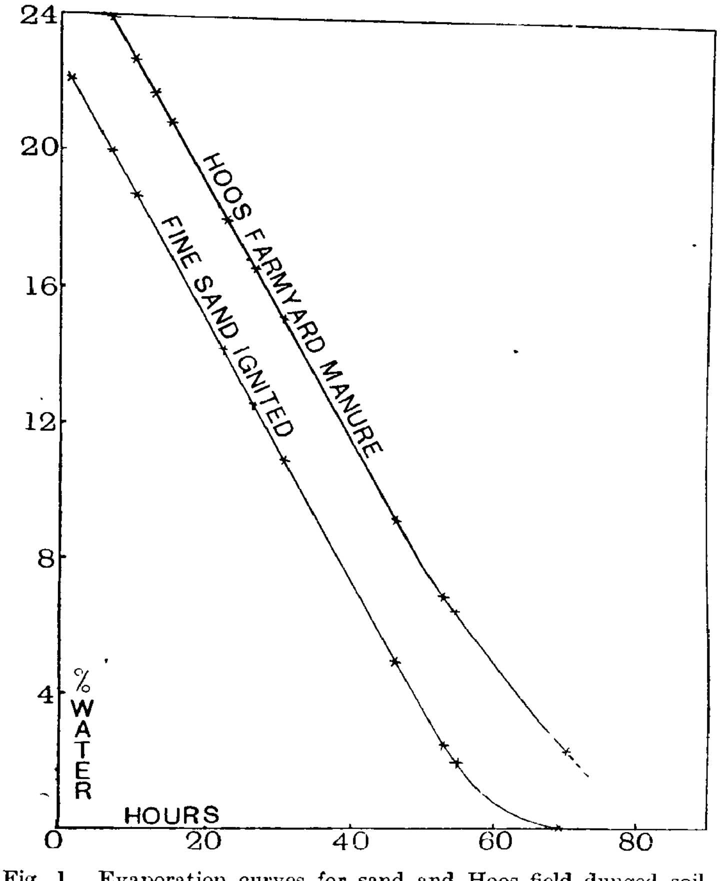

Fig. 1. Evaporation curves for sand and Hoos field dunged soil, using Patten and Gallagher's method.

of soil introduces certain complications which react on the evaporation. Again the temperature of the soil changes during the weighing, and evaporation is suspended every time the bottles are removed for this purpose.

Description of the apparatus used.

In the first experiments a very thin layer of soil held in a pan of wire gauze was suspended over sulphuric acid to allow evaporation to take place uniformly from all round the surface of the soil particles, but this method was abandoned as the effect of the temperature changes was much too serious. The following apparatus was then devised in which the moist soil could be weighed *without removing it from the evaporation chamber*, and therefore without disturbing its temperature.

A short glass tripod rests on the bottom of a rectangular glass vessel containing 50 c.c. of sulphuric acid of known strength. About 5 grams of moist soil is placed in a thin layer on a square pan of wire gauze, 1 cm. below the bottom of which is attached an aluminium base plate to catch any dried particles which may fall through. The pan of moist soil, together with a stout copper holder, is placed on the tripod. The top of the glass vessel is covered with four glass plates (Fig. 2).

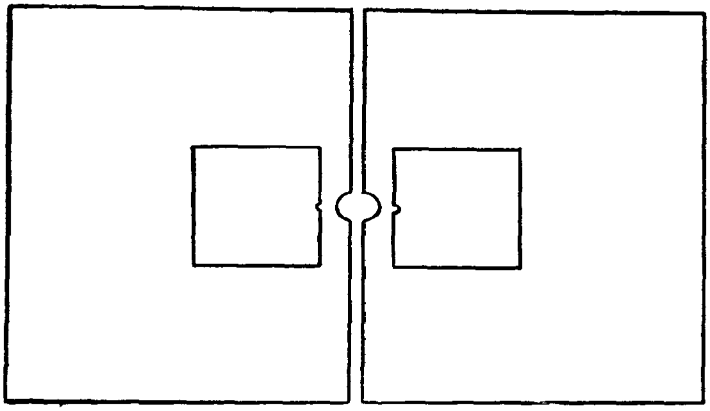

Fig. 2. Glass plates forming cover of evaporation cell.

The two larger ones are made by splitting a plate of slightly greater area than the top of the vessel across the diameter of a $\frac{1}{2}$ cm. hole bored in its centre. The copper holder passes through the hole and terminates in a hook. The hole is closed by the two smaller glass plates bored and split in the same way as the larger ones, the hole being made this time of the same diameter as the wire of the copper holder. All the edges in contact are coated with vaseline. The evaporation cell (Fig. 3) thus constructed is found by tests to be air-tight for all practical purposes and no measurable amount of atmospheric water vapour is absorbed by the concentrated sulphuric acid even after fourteen days.

A cylindrical zinc water bath holds six of these cells arranged radially. The bath is mounted on a circular rail resting on wheels attached to the base plate of the apparatus and is kept at a constant temperature of 24·2° C. by the usual arrangements. A table supporting a balance spans the front of the bath, which can be rotated on its rail so that any one of the evaporation cells is under the balance. A vertical copper wire swinging from the bottom of one balance pan passes through

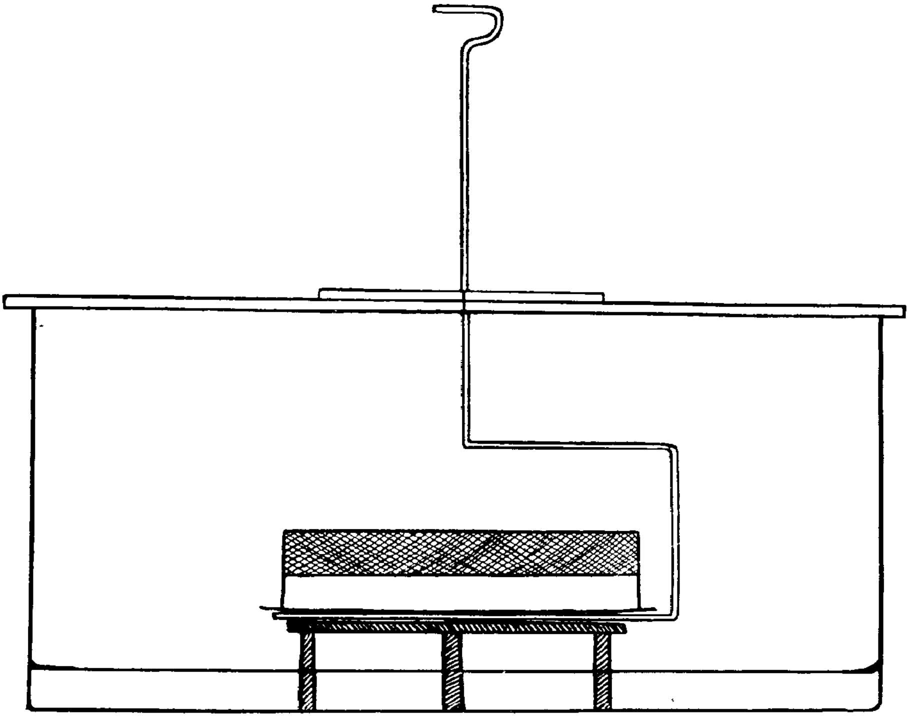

Fig. 3. Evaporation cell showing tray of soil resting on glass tripod and provided with hook for weighing.

the base of the balance and the table, and terminates in a small hook. A general view of the complete apparatus is shown in Fig. 4.

The pan of moist soil is weighed by sliding the two smaller glass plates apart, and thus exposing the larger hole in the plates, lifting up the pan by its holder, and attaching this to the hook of the suspended wire. Weighings are made to the nearest milligramme and the whole operation takes less than two minutes. The apparatus works very satisfactorily and its efficiency can be tested as follows:—The soil is moistened with dilute ammonia and hydrochloric acid is put into the

rectangular vessel. The glass covers are then placed in position, and when the resulting air disturbances have died down, the evaporation

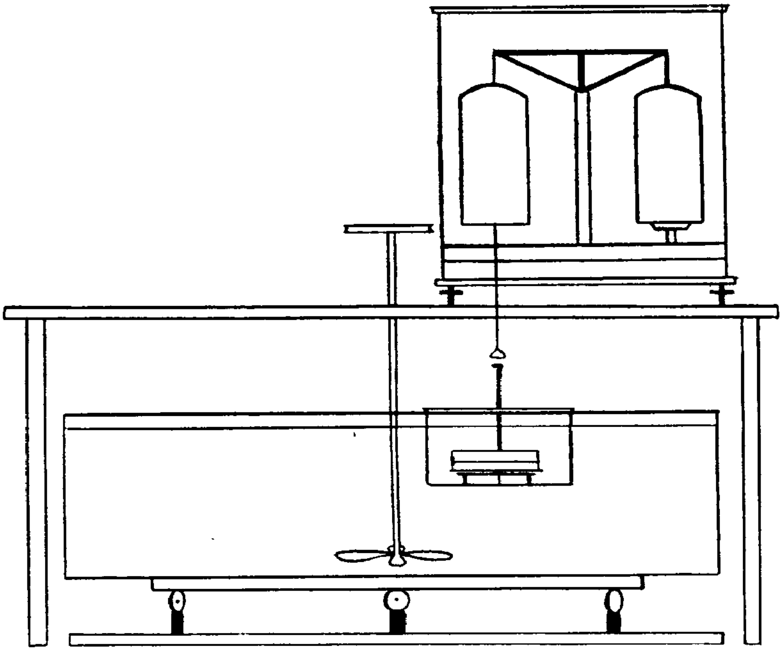

Fig. 4. View of whole apparatus (not drawn to scale).

of the water vapour is clearly shown by the formation of ammonium chloride, which travels in well-defined paths from the top of the soil

Hoos Field Dunged Plot (*No.* 7²) (*taken moist from field*).

26/11/13						27/11/13		
Time	Wt. in grms.	Percent. of moisture left in soil	Time	Wt. in grms.	Percent. of moisture left in soil	Time	Wt. in grms.	Percent. of moisture left in soil
A.M.			P.M.			A.M.		
11.52	25·344	26·43	2.37½	24·440	5·31	10.18	24·215	·05
P.M.			3.1	·374	3·76	P.M.		
12.12	·191	22·86	3.37	·304	2·13	6.30	·213	0
12.29½	·068	19·98	4.0	·280	1·57			
12.53	24·915	16·41	4.49	·255	·98			
1.12½	·800	13·72	5.21	·247	·79			
1.29	·711	11·64	6.4	·241	·65			
1.49	·616	9·42	6.51	·237	·56			
2.8	·539	7·62	9.44½	·228	·35			

Final dry wt. of soil 4·279 grms.

No loss on heating in steam oven

and streams out from between the aluminium base plate and the bottom of the soil pan.

Whether soil or sand is being used the experimental points always lie exactly on a smooth curve. For this reason and for the sake of clearness, the experimental points have been omitted in the accompanying curves. The values obtained in a typical experiment are given on page 462. If these points are plotted they are found to lie exactly on a smooth curve.

Experimental methods.

Certain manipulative difficulties have to be overcome before agreement of duplicate experiments can be obtained. Where possible the soil is used moist from the field, taken with a 1 in. augur, and passed through a 3 mm. sieve, but not compressed in any way. It is then placed in tightly corked bottles and weighed out as required. When either air-dried soil or a soil fraction is used it is spread in a thin layer on a glass plate, moistened by spraying it with an atomiser and turned at frequent intervals with a spatula. When thoroughly moist it is placed over water in a closed vessel for at least 24 hours in order to ensure uniform distribution of the water film.

For convenience experiments are usually done in duplicate and the amount of moist soil used in any one experiment contains approximately about 1 gramme of water and gives a final dry weight of 4·2–4·4 grammes. When the pan of moist soil is placed in the apparatus it is left for some time to gain the temperature of the bath before weighings are begun. Weighings are made at intervals of about 15 minutes until most of the moisture has evaporated, when they are taken less frequently. When constant weight is reached the soils are rapidly transferred to weighing bottles and heated in a steam oven for 24 hours at 100° C. in order to obtain the final dry weight of the soil. A certain number of experiments have been made in which the soil was heated in vacuo for 24 hours at 100° C. Important differences have been obtained in the two methods, and are being further investigated. The former method of drying has been used exclusively in the present paper. The experimental curves show the relation between the time (in hours) and the percentage of water (calculated on the final dry weight). In every case they are mean curves, obtained by combining a considerable number of experimental curves which agree closely among themselves.

Description of soils and soil fractions used.

A considerable number of soils and soil fractions have been experimented upon, and the following will be considered in the present paper:

(A) *Soil fractions.*

(1) "Fine Sand" (0·2 to 0·04 mm. diameter) both ignited and unignited.

(2) "Silt" (0·04 to 0·01 mm. diameter) both ignited and unignited.

(B) A sample of pure china clay kindly sent us by Dr Mellor.

(C) *Soils.* Two soils were used (1) Hoos field dunged soil from plot receiving 14 tons of farmyard manure annually, (2) a garden soil. Both were used as taken moist from the soil. The mechanical analysis is as follows:

	Hoos field farmyard manured soil (top 9″)	Garden soil
Fine gravel....	1·8 %	7·1 %
Coarse sand ...	7·9	12·8
Fine sand	25·3	23·4
Silt...........	21·0	17·0
Fine silt	11·0	8·4
Clay..........	15·7	13·7
Ignition loss..	11·7	12·8

Discussion of results.

1. *Sand and Silt.*—The curves showing the evaporation from fine sand (ignited) and Hoos field farmyard manured soil over concentrated sulphuric acid are shown in Fig. 5, curves *A* and *B* respectively. These should be compared with Fig. 1, which gives the curves for evaporation from the same materials, by Patten and Gallagher's method. The comparison shows that the latter method is not reliable even for qualitative results. Referring again to Fig. 5, general inspection indicates that the curves are continuous over the whole range and have no break of any kind, nor any sudden change of direction. There can thus be no abrupt change in the physical state of the water between the limits experimented upon. The experimental points for fine sand from the initial percentage down to about 10 % lie exactly on a straight line,

i.e. evaporation is linear down to this point. Below 10 % the curve begins to bend slightly to the right, until less than 0·4 % of water remains, when there is a comparatively sharp bend and the curve becomes parallel to the time axis, and a short distance above it. Unfortunately the scale of the curve has had to be reduced for reproduction purposes, and the details given above require a larger scale in order to be shown clearly. Curves of the same type are given by silt (ignited) which is linear down to about 12 %, and by ignited garden soil which is linear down to about 14 %. Experiments done with non-ignited fine sand, and silt, give results which do not differ appreciably from those given by these fractions after ignition.

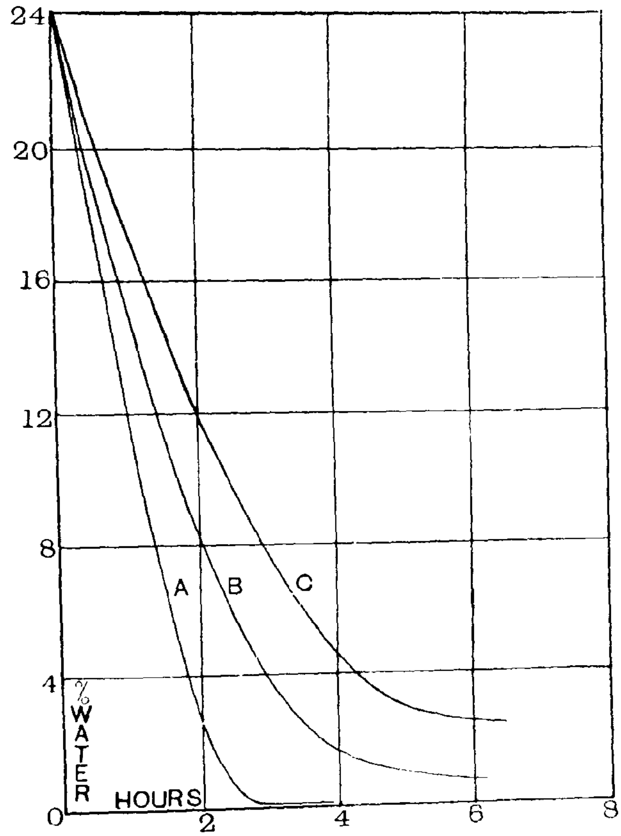

Fig. 5.

Curve A. Evaporation from fine sand (ignited) over concentrated H_2SO_4.
" B. " " Hoos field soil (dunged plot) over conc. H_2SO_4.
" C. " " " " " 55·4 % H_2SO_4.

2. *Soils.* In the case of soils (curve *B*, Fig. 5) the rate of evaporation constantly decreases, and is always less than that for sand. But it is comparatively rapid until the water is reduced to about 4 %. Then the curve bends over and becomes gradually more nearly parallel to the time axis, which it touches after about 30 hours. The curve is everywhere concave upward. There is no indication of any linear relation in the initial stages of evaporation. Had the water content been increased until the soil was waterlogged this initial linear portion might of course appear. The curvature although slight, is quite definite and can be relied upon because of the accuracy of the experimental points. In order to study the evaporation under less intense conditions and also to obtain more information about the bend at 4 % a series of experiments was done over weaker sulphuric acid, the strength being so chosen that when equilibrium was reached the soil would still contain about 2 % of water. The curve is shown in *C*, Fig. 5. It is obviously of the same type as curve *B*. The probability is therefore that the bend at 4 % noticed in curve *B* is due primarily to the small percentage of water present and not to any change in its physical state. It is obvious from Fig. 5 that soil behaves in a very different manner from sand. In spite of the much greater surface it presents evaporation from soil is slower than from an equal weight of sand.

The Influence of Humus. Some of the garden soil was divided into three portions: *A* was ignited at a dull red heat and remoistened; *B* was air-dried and treated with acid and then with 2 % caustic soda to remove humus, it was then washed, air-dried, and remoistened; *C* was untreated, being simply air-dried and remoistened by the atomiser. Evaporation experiments were made and the curves are shown in Fig. 6, *A*, *B* and *C*.

The removal of the humus makes comparatively little difference; evaporation is a trifle more rapid than in the case of the untreated soil.

The Influence of the Colloids. Ignition entirely alters the character of the curve, making it precisely similar to that given by sand or silt. Evaporation is linear down to 14 %, then the curve begins to bend slightly until the water reaches about 5 % when there is a sharp bend[1]. Although ignition entirely alters the chemical properties of a soil it does not at any rate do away with all the fine particles. If a bottle of ignited soil be shaken a cloud of fine particles remains suspended in the air, and on the removal of the stopper, floats away like

[1] This curve has not been drawn above 11 % in the diagram, as the portions *A* and *B* were only moistened up to 12 %—14 %.

smoke. Clay which has been separated from the soil by mechanical analysis and then air-dried, behaves in the same manner.

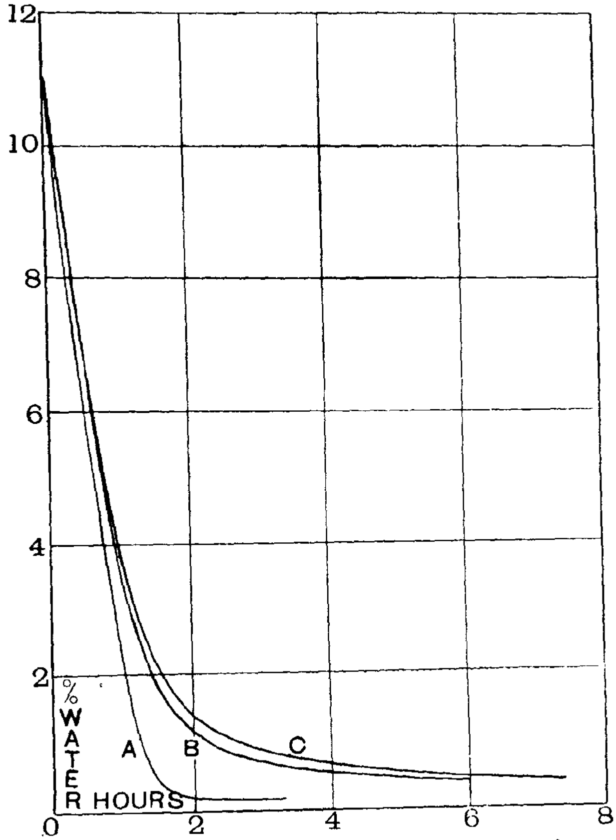

Fig. 6. Evaporation over concentrated H_2SO_4 of:
(A) Garden soil (ignited).
(B) ,, extracted with 2 % caustic soda.
(C) ,, untreated.

It follows then that ignition of soil, which destroys the colloidal nature of the clay, changes the type of the evaporation curve making it identical with the sand curves. The inference from these experiments is that the colloidal nature of the clay is mainly responsible for the characteristic shape of the evaporation curve from soil.

Further confirmation of this view is obtained from experiments on china clay. The evaporation curve is practically identical with that given by fine sand (ignited). The two curves are almost indistinguishable when plotted together, the china clay evaporation being a trifle the

slower of the two. This china clay possesses only very feeble colloidal properties. The rate of sedimentation in a dilute acid solution is no faster than in distilled water, and ammonia produces very little deflocculating effect.

Summarising the evidence from the experimental curves we can state that soil behaves in a different manner from sand, silt, or china clay. But *ignited* soil does not. Therefore the factor (or factors) causing the difference is lost on ignition. The soluble humus is not responsible for the characteristic nature of the evaporation from soil. Again, ignition of the fine sand or silt makes no appreciable difference to the evaporation from these fractions. We may infer that the factors can only be in the clay, or the insoluble organic matter. We should not expect this latter to be any more effective than the soluble humus, and moreover the whole of the organic matter of the sand or silt fractions is destroyed by ignition without affecting the resulting evaporation curve. Hence the clay fraction probably contains the controlling factor. Now china clay does not contain this factor for its evaporation closely approximates to that of sand. It also differs from the "clay" fraction obtained from soil, in that its colloidal properties are far less marked. It must be concluded then, that the colloidal properties of the clay are the prime cause of the differences noted above.

Examination of the rate curves.

The main points brought out by an inspection of Figs. 5 and 6 are indicated above. There is, however, a far more sensitive method of examination,—to study the *rate* of evaporation, or the first differential of the experimental curves. The rate curve is obtained in the usual way, by drawing the experimental curves on a large scale, measuring the tangent $\left(\frac{dw}{dt}\right)$ at various points, and then plotting the tangent against the corresponding percentages of water (w). When the rate of evaporation is very rapid or very slow, *i.e.* at the beginning and end of the experimental curves, the measurement has to be done with care. These rate curves are plotted in Fig. 7. Curve *I* refers to Ignited Garden Soil, and shows the course of the evaporation in a striking manner. From initial percentages down to about 14 % the rate is constant. Over this region evaporation is proceeding as it would from a dish of water, *i.e.* a free water surface. Then from 14 % to about 2 % the rate decreases linearly. In other words, over this range a pro-

portionality holds between the rate of evaporation and the water content, thus giving an experimental confirmation of the deductions made from considerations of diffusion by Leather[1] from a paper by Briggs[2]. Over this region the following equation obviously holds:

$$-\frac{dw}{dt} - A = Bw \qquad \text{.................... (1)},$$

where A and B are constants; $\frac{dw}{dt}$ = rate of evaporation; w = percentage of water.

Integration of this equation gives

$$\log_e (Bw + A) = -Bt + C, \quad \text{where } C = \text{constant},$$

or

$$(Bw + A) = e^{-Bt+C} \qquad \text{.................. (2)},$$

which is the equation to the *experimental* curve (Fig. 6, Curve A) over the range 14 %—2 %. The equation is an expression of the diffusion of water vapour and is consequently exponential in type. Below 2 % the rate falls off rapidly to zero. As Briggs has shown, it is in this region of low percentages that the water is mainly in the interstitial spaces between the grains. We should also expect the evaporation of the last traces of water to be somewhat irregular. In all probability the rate curve over this region is the complex expression of these facts.

It is essential to understand that the deductions of Cameron, Briggs, Buckingham and Leather are based on the assumption that the soil may be regarded as a group of mineral particles over which the water is distributed in the form of a continuous film. Ignited soil, sand, and silt fulfil this definition, and the conclusions of these investigators are here verified experimentally as far as evaporation from these materials is concerned. But the methods used in the present investigation enable us to refine on their first generalisations and to bring out the differences between an actual soil and such a group of mineral particles as they postulate.

As mentioned above, the points of dissimilarity are well brought out in the rate curves. Curves II and III, Fig. 7, refer to Hoos Field Dunged Soil over concentrated and 55·4 % sulphuric acid respectively. In the first place the similarity of these two curves confirms the statement on page 466, that the bend in the experimental curve for soil at 4 % is due only to the small percentage of water in the soil and

[1] Leather, *Memoirs Agric. Dept. Pusa.* Chem. Series, Vol. I. No. 6.
[2] Briggs, U. S. Dept. of Agric. Bureau of Soils, Bulletin No. 10.

is no indication of a change in the physical state of the water. There is no sudden decrease in the rate of evaporation and hence, for this soil, no confirmation of the results obtained by Cameron and Gallagher[1] who find a rapid decrease in the rate of evaporation at a certain percentage of water, designated the "optimum water content."

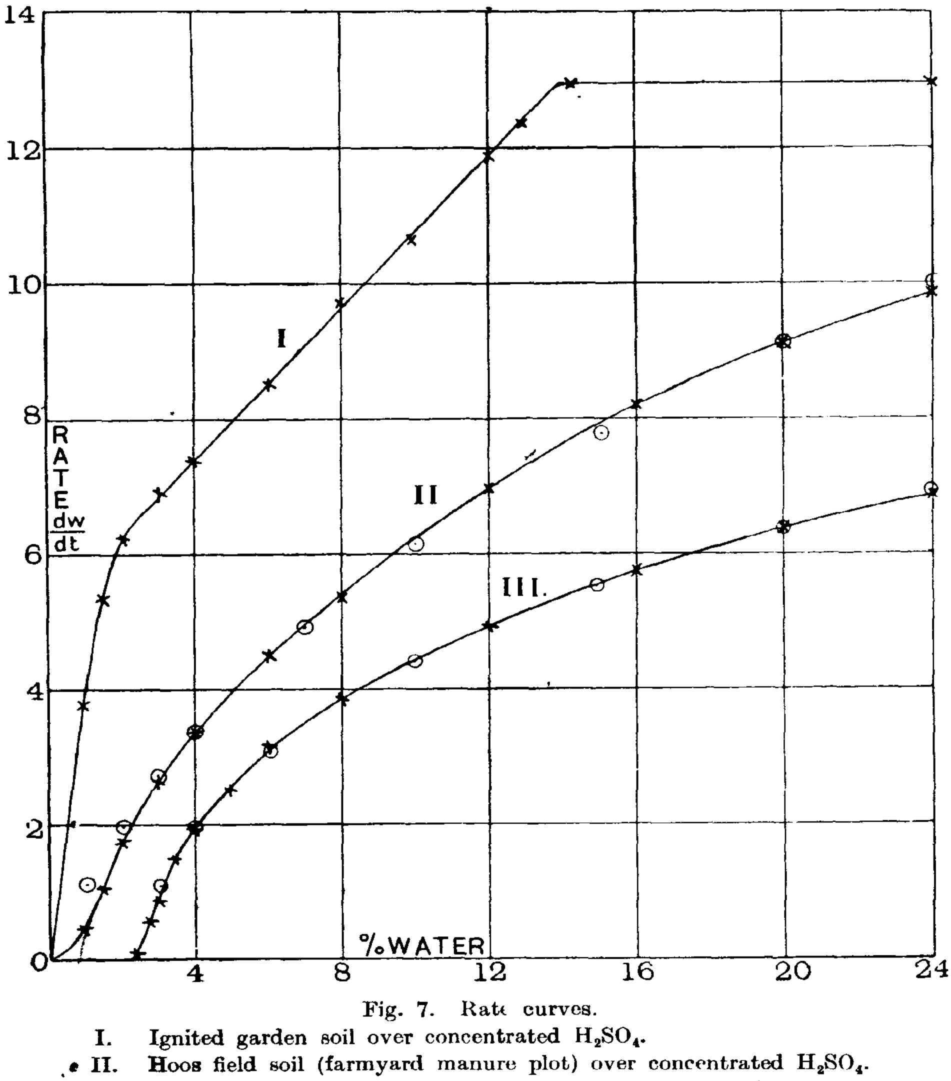

Fig. 7. Rate curves.

I. Ignited garden soil over concentrated H_2SO_4.
II. Hoos field soil (farmyard manure plot) over concentrated H_2SO_4.
III. " " " " 55·4 % H_2SO_4.

[1] U.S. Bureau of Soils, Bulletin 50.

The rate curve for soil is nowhere linear and thus the simple equation (1) does not apply. But as diffusion of the water vapour must still take place under the influence of the vapour pressure, the inference is that the relation between moisture content and vapour pressure is more complex than in the case of sand or silt. Judging from the shape of the rate curves they would seem to be satisfied by an exponential equation, *e.g.*

$$w = Ke^{A\frac{dw}{dt}},$$

where K and A are constants, w = percentage of water by weight and $\frac{dw}{dt}$ = time rate of decrease of this percentage. Before this equation can be applied to the rate curves it has to be slightly modified. As it stands, it implies that when $\frac{dw}{dt} = 0$, the right-hand side has the value K, *i.e.* $w = K$. But we know from the curve that when $\frac{dw}{dt} = 0$, $w = 0$ also. If we introduce K into the left-hand side as well we obtain

$$(w + K) = Ke^{A\frac{dw}{dt}} \quad \text{..................(3).}$$

In this equation, when $\frac{dw}{dt} = 0$, $w = 0$[1].

For our present purpose, a more convenient form of (3) is obtained by taking the logarithm of each side:

$$\log_e (w + K) = \log_e K + A\frac{dw}{dt},$$

or

$$2{\cdot}303 \log_{10} (w + K) - \log_e K = A\frac{dw}{dt} \quad \text{..........(4).}$$

But this equation does not exactly fit the rate curves although it is of the same general type. This discrepancy shows that equation (4) is not a complete expression of the case but that some other factor comes into play and must be introduced into the equation.

This factor is an expression of the effect of the *surface* on the rate of evaporation. Consider an average-sized particle covered with a film of water. It is immaterial for our present purpose whether this coating be regarded as colloidal, or as a film of free water, or both. Evaporation takes place from the surface of this film and its thickness

[1] The addition of K to the left-hand side only means that the origin is moved a distance K in the negative direction of w.

gradually diminishes. Thus the *surface* from which evaporation can take place also diminishes as the coating shrinks in size[1]. This conception is introduced into the above equation in the following manner.

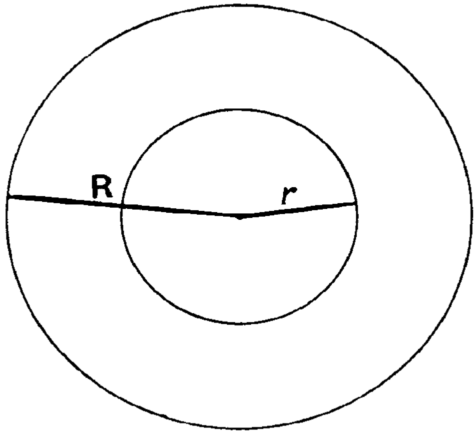

Fig. 8.

For simplicity let the average-sized particle of radius r be covered with a film of water whose radius is R (Fig. 8). Now it has been shown theoretically by Stefan and experimentally by Winklemann[2] that evaporation is proportional to the *linear* dimensions of the surface, and not as would at first be expected, to the *area* of the surface. Applying this to the present case we have

$$\frac{dw}{dt} = C\sqrt{4\pi R^2}, \text{ where } C = \text{constant},$$

or

$$\frac{dw}{dt} = C'R \quad\ldots\ldots\ldots\ldots\ldots\ldots(5),$$

$$C' = C\sqrt{4\pi} = \text{constant}.$$

Now the percentage of water (w) by weight is

$$\frac{\text{wt. of water}}{\text{wt. of soil}} = \frac{\frac{4}{3}\pi (R^3 - r^3) \times 100}{\frac{4}{3}\pi r^3 s} = w.$$

where s = specific gravity of the soil. Hence

$$R^3 = r^3\left(\frac{sw}{100} + 1\right),$$

or

$$R = r\sqrt[3]{\left(\frac{ws}{100} + 1\right)} \quad\ldots\ldots\ldots\ldots\ldots(6).$$

[1] This effect has been recognised by van Bemmelen. See *Die Absorption*, p. 275.

[2] Quoted in Preston's *Heat*, 2nd Edition, p. 357.

Substituting the value of R from (6) in (5),

$$\frac{dw}{dt} = C'r \sqrt[3]{\left(\frac{ws}{100} + 1\right)} = D \cdot \sqrt[3]{\left(\frac{ws}{100} + 1\right)} \quad \ldots\ldots\ldots (7),$$

where $D =$ constant.

A similar expression will hold for every soil particle, so that the total effect should be obtained by a kind of summation. However, if we give $\frac{dw}{dt}$ and w the above meanings, it can be assumed with sufficient accuracy that equation (7) gives the total surface effect. No account is here taken of the well-known fact that the vapour pressure over a curved surface differs from that over a plane surface, for this difference is only appreciable when the curvature of the film is very great, *i.e.* at exceedingly low percentages of water in the present case.

Under the experimental condition that $\frac{dw}{dt} = 0$ when $w = 0$, the only possible combination of equations (4) and (7) is

$$\frac{A}{D} \cdot \frac{dw}{dt} = \sqrt[3]{\left(\frac{ws}{100} + 1\right)} [2{\cdot}303 \log_{10} (w + K) - \log_e K] \quad \ldots (8),$$

or finally, taking $s = 2{\cdot}5$ and writing $B = \frac{A}{D}$,

$$B \frac{dw}{dt} = \sqrt[3]{\left(\frac{w}{40} + 1\right)} [2{\cdot}303 \log_{10} (w + K) - \log_e K] \quad \ldots (9).$$

To evaluate the constants B and K, a method of successive approximation is necessary, owing to the presence of K in the term $\log_{10} (w + K)$. This is effected as follows:

The term $\log_{10} (w + K)$ is replaced by $\log_{10} w$, and in equation (9) thus amended, are substituted the values $\left(w = 20, \frac{dw}{dt} = 9{\cdot}07\right)$ and $\left(w = 4, \frac{dw}{dt} = 3{\cdot}35\right)$ taken from Curve II, Fig. 8. The two simultaneous equations thus obtained are solved for B and $\log_e K$, which have the following values:

$$B = {\cdot}343; \ \log_e K = {\cdot}272, \text{ from which } K = 1{\cdot}3125.$$

This value for K is substituted in the term $\log_{10} (w + K)$ of equation (9), and the equations again solved for B and $\log_e K$, as above. The new value of K thus obtained replaces the former one and the process is repeated until the values of B and $\log_e K$ change only slightly at each further approximation. The final values obtained are

$$B = {\cdot}2515; \ \log_e K = 1{\cdot}1495; \ K = 3{\cdot}1557.$$

Hence the complete equation is

$$\cdot 2515 \frac{dw}{dt} = \sqrt[3]{\left(\frac{w}{40} + 1\right)} [2\cdot 303 \log_{10}(w + 3\cdot 1557) - 1\cdot 1495] \ldots (10).$$

A similar equation holds for Curve III, Fig. 8, which is the rate curve for evaporation from Hoos Field Dunged Soil over weaker acid. Here the equilibrium percentage of water in the soil is 2·2. Hence before applying equation (9) above, to this curve, the value 2·2 has to be everywhere subtracted from w. The calculation of B and $\log_e K$ is carried out in exactly the same way as before, and leads to the following values:

$$B = \cdot 506;\ \log_e K = \cdot 1278;\ K = 1\cdot 1363.$$

The complete equation then becomes

$$\cdot 506 \frac{dw}{dt} = \sqrt[3]{\left(\frac{w}{40} + 1\right)} [2\cdot 303 \log_{10}(w - 2\cdot 2 + 1\cdot 1363) - \cdot 1278] \ldots (11).$$

The calculated points given by equations (10) and (11) are shown in Fig. 7, by circles, and the experimental points through which the rate curves are drawn, by crosses. It will be seen that excellent agreement holds except at very low percentages, where some divergence would be expected, even if only that due to experimental irregularities. Otherwise, this equation completely defines the rate curves over the range of these experiments. Thus the rate of evaporation at any given percentage is completely given by two factors—one expressing the effect of the gradually diminishing surface, and the other giving an empirical measure of the influence of the vapour pressure.

Comparison with the results obtained with sand shows how wide is the difference in evaporation from the two materials. One cause—the effect of surface—has been discussed above. The other cause, represented in equation (9) by an empirical expression, is intimately connected with the relation between the water content and the vapour pressure. All the evidence in the present paper goes to show that this relation is bound up with the colloidal properties of the clay.

SUMMARY.

The evaporation of water from the soil fractions "sand" and "silt," from china clay, and ignited soil is a relatively simple phenomenon which can readily be explained by the known laws of evaporation and diffusion. The evaporation from soil is more complex, something being present which operates in making the relation between the soil and the soil

water of a different and closer nature than in the case of sand, etc. The effect is not due to the soluble humus, for the removal of this material from the soil does not appreciably affect the phenomena of evaporation. Any possible effect of the insoluble organic matter is largely eliminated by the consideration that *ignited* sand and silt behave like the *unignited* material.

But when the colloidal properties of the soil fraction "clay" are destroyed, the evaporation curve is completely altered, and becomes identical with that given by "sand," or "silt." Again, evaporation from china clay, which shows very feeble colloidal properties, is of the same character as that from sand. We may infer then, that the colloidal properties of the clay fraction are in part, if not mainly, responsible for the characteristic shape of the evaporation curve from soil.

Further information on the process of evaporation has been obtained by a mathematical examination of the rate curves for soil. Two factors have been distinguished, which operate over practically the whole range of water content dealt with in these experiments. In the first place the simple linear relationship observed with sand is not seen with soil, the curve being more nearly exponential in character. This indicates that the relationship of water to soil is quite different from its relationship to sand, a circumstance which has been traced as already stated to the colloids. This relationship has only been expressed empirically but it is probably connected with the relation between vapour pressure and moisture content. There is clearly something else at work for the curve is not of the simple exponential type; it is necessary to allow for another factor: the effect on evaporation of the decreasing water surface in the soil—the surface obviously diminishing as evaporation continues.

The equation finally developed is

$$A\frac{dw}{dt} = \sqrt[3]{\left(\frac{ws}{100}+1\right)}\left[2{\cdot}303\log_{10}(w+K) - \log_e K\right].$$

where $\frac{dw}{dt}$ = rate of evaporation.

w = percentage of water present by weight.

s = specific gravity of the soil.

A and K = constants.

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LIME-SULPHUR SPRAYS, THEIR COMPOSITION AND ANALYSIS.

By A. A. RAMSAY.

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THE analysis of a sample of manufactured lime-sulphur mixture "A" was made according to the methods set out in the *Journal of Agricultural Science*[1]. The following results were obtained, the amount being stated in grams per 100 c.c.

Specific gravity 1·3735.

Monosulphide sulphur	8·30	associated with calcium = lime (CaO)	14·53
Polysulphide „	30·47		
Thiosulphate „	0·99	„ „ „ = „	0·86
Sulphate and sulphite sulphur ..	0·07	„ „ „ = „	0·11
Total sulphur	39·83		15·50
The actual determinations were..	39·83	and	15·48

It was noted in previous work that a portion of the so-called "Polysulphidal" sulphur was loosely held and was soluble in benzene. Time was not available then to investigate the matter, but this point has since been looked into.

Carbon Bisulphide naturally suggested itself as the most suitable solvent provided there was no decomposition of the solvent itself. The idea occurred that even were calcium thiocarbonate produced it would be soluble in the aqueous portion and insoluble in carbon bisulphide.

50 c.c. of the concentrated lime sulphur diluted to 500 c.c. as used for the analysis were made up, and of this aliquots of 10 c.c., 20 c.c. and 30 c.c. were shaken up with carbon bisulphide, using first 10, 20, and 30 c.c. carbon bisulphide, second another 10, 20 and 30 c.c., third 5, 10 and 15 c.c., fourth 5, 10 and 15 c.c. respectively.

[1] "The preparation and composition of Lime-Sulphur Sprays," by Ramsay, *Journal of Agricultural Science*, VI. pt. II. p. 194.

On weighing the sulphur obtained and calculating to the original concentrated mixture the same figure for soluble sulphur was obtained.

Three further quantities of 10 c.c. diluted lime-sulphur mixture were again taken and diluted with water to 20, 25, and 30 c.c. respectively, and extracted as described above with carbon bisulphide. On weighing the sulphur and calculating back the same figure for soluble sulphur was obtained.

The lime-sulphur solution from which soluble sulphur had been extracted by carbon bisulphide was examined for the presence of thiocarbonates but negative results were obtained.

I have, therefore, decided to adopt the following method for the determination of "Free Sulphur."

10 c.c. of the mixture prepared for analysis (50 c.c. concentrated diluted to 500 c.c. with water) are repeatedly shaken with two lots of 10 c.c. each and two lots of 5 c.c. each of carbon bisulphide and the carbon bisulphide collected in a tared Erlenmeyer flask of about 150 c.c. capacity. The bisulphide is driven off by placing in a bath of warm water and the flask dried to constant weight on top of the air oven or at a temperature not exceeding 70° C.

The results obtained on three quantities of 10 c.c. of lime sulphur mentioned above were 0·2310, 0·2312, 0·2311, giving a mean determination of 0·2311.

The so-called "polysulphidal" sulphur, therefore, consists of

Free sulphur	23·11
Combined sulphur (*i.e.* sulphidal S.)	7·36
"Polysulphidal" sulphur ..	30·47

We therefore have 8·30 + 7·36 = 15·66 sulphur associated with calcium equivalent to 14·53 lime (CaO). The remaining sulphur and lime are all accounted for.

Let us consider for a moment the sulphide and hydrosulphide compounds which would probably be present in lime-sulphur solutions.

1. *Calcium monosulphide, CaS.* This compound could not exist as such, on account of its unstableness in water, in which it is only sparingly soluble but suffers decomposition. The reactions which would probably occur are represented by the following equations:

$$2CaS + 2H_2O = Ca(HS)_2 + CaO \, . \, H_2O,$$
$$Ca(HS)_2 + H_2O = CaHSOH + H_2S,$$
$$CaH_2O_2 + H_2S = CaS + H_2O,$$

completing the cycle. Or

$$CaS + H_2O = Ca \,.\, HS \,.\, OH$$

and
$$CaH_2O_2 + H_2S = CaHSOH + H_2O.$$

This compound, the hydroxyhydrosulphide, has been shown to be a constituent of lime-sulphur solutions[1].

2. *Calcium disulphide, CaS_2.* This is a very likely component, as it is permanent when in solution in water.

$$3CaH_2O_2 + S_6 = 2CaS_2 + CaS_2O_3 + 3H_2O.$$

3. *Calcium pentasulphide, CaS_5.* This might primarily be formed, but would not be likely to persist, since although CaH_2S_2 dissolves sulphur forming CaS_5 the action is reversible.

$$3CaH_2O_2 + S_{12} \rightleftarrows 2CaS_5 + CaS_2O_3 + 3H_2O.$$

Odling and Divers[2] state that H_2S decomposes polysulphides of lime; H_2S is liberated when CaH_2S_2 is boiled with S; boiling alone will liberate H_2S, but with S dissolving H_2S is increased. Again H_2S passed into CaS_5 solution precipitates S as freely as it would arsenic or antimony.

$$CaS_5 + H_2S = CaH_2S_2 + S_4$$

and
$$CaH_2S_2 + S_4 = CaS_5 + H_2S.$$

Divers also states that CaS_5 is decomposed by boiling its solution into $CaO, \cdot H_2S$ and S.

Again, although it is generally represented, that CaS_5 is converted into thiosulphate and sulphuretted hydrogen, in accordance with the equation $CaS_5 + 3H_2O = CaS_2O_3 + 3H_2S$, Divers does not agree with this but thinks the equation $CaS_5 + O_3 = CaS_2O_3 + S_3$ represents the reaction which takes place.

For these reasons I think it unlikely that calcium pentasulphide is present.

The most probable sulphides, therefore, appear to be the hydroxyhydrosulphide, CaHSOH, which may be considered as a monosulphide, for the ratio of calcium to sulphur is as Ca : S, and the disulphide CaS_2.

If the above conclusions are correct, it is a simple matter to calculate the amounts of hydroxyhydrosulphide and disulphide present, from the figures obtained by analysis giving 15·66 sulphur in combination with calcium equivalent to 14·53 lime.

[1] "The preparation and composition of Lime Sulphur Sprays," by Ramsay, *Journal of Agricultural Science*, VI. pt. II. p. 201.

[2] "On Calcium Sulphides," by Divers, *Journal Chemical Society*, 1884, p. 270.

The results obtained by calculation are

Hydroxyhydrosulphide	0·944	sulphur associated with calcium equivalent to	1·653 lime
Disulphide	14·716	„ „ „ „	12·877 „

We are now able to complete the statement of analysis of the concentrated lime-sulphur mixture, *viz.*:

	sulphur		lime
Hydroxyhydrosulphide containing	·944	associated with calcium equivalent to	1·653
Disulphide	14·716	„ „ „	12·877
Free sulphur	23·11		
Thiosulphate	0·99	„ „ „	0·86
Sulphate and sulphite ..	0·07	„ „ „	0·11
Total	39·83		15·50

Further corroboration of the above was afforded by weighing the sulphur liberated by the $\frac{N}{10}$ iodine solution in the determination of the so-called "monosulphide" sulphur according to the following equation:

$$CaS_x + I_2 = CaI_2 + S_x.$$

The mean of four determinations on 25 c.c. of the mixture as prepared for analysis, gave 0·9777 grams sulphur, equivalent to 39·11 grams sulphur per 100 c.c. of the original lime-sulphur spray.

This figure should have been ·944 plus 14·716 plus 23·110 = 38·77.

The sulphur precipitated by iodine is therefore slightly high, which may be due to occluded iodine.

The analytical data obtained upon the examination of another manufactured lime-sulphur mixture "B" are now given below—the results being stated in grams per 100 c.c.

			lime
Monosulphide sulphur	6·88	associated with calcium equivalent to	12·04
Polysulphide „	26·51		
Thiosulphate „	1·62	„ „ „	1·41
Sulphate and sulphite sulphur	·10	„ „ „	·17
			13·62
Total sulphur	35·11		
Free sulphur	19·88		

We have therefore 6·88 + (26·51 − 19·88) = 13·51 sulphur in combination with calcium equivalent to 12·04 lime as hydroxyhydrosulphide and disulphide.

By calculation we obtain

	sulphur		lime
Hydroxyhydrosulphide	·249	associated with calcium equivalent to	·436
Disulphide	13·261	„ „ „	11·604
	13·51		12·04

We therefore obtain the following results:

	sulphur		lime
Hydroxyhydrosulphide	·249	associated with calcium equivalent to	·436
Disulphide	13·261	,, ,, ,,	11·604
Free sulphur	19·88		
Thiosulphate	1·62	,, ,, ,,	1·41
Sulphate and sulphite ..	·10	,, ,, ,,	·17
	35·11		13·62

An examination was now made of a lime-sulphur solution "C," prepared by boiling together lime, sulphur and water. As has been shown in a previous paper[1], these boiled mixtures differ from the manufactured and imported concentrated lime-sulphur solutions, in that the former contain a very much greater quantity of thiosulphate, and consequently less so-called "polysulphide" sulphur.

The data obtained are stated below, the results being expressed in grams per 100 c.c. as formerly.

			lime
Monosulphide sulphur	3·30	associated with calcium equivalent to	5·78
Polysulphide ,, ...	11·55		
Thiosulphate ,, ...	3·78	,, ,, ,,	3·31
Sulphate and sulphide sulphur	·09	,, ,, ,,	·15
Total sulphur	18·72		9·24
		Actual (gravimetric) determination of total lime gave	9·21
Free sulphur	8·61		

We have therefore $3{\cdot}30 + (11{\cdot}55 - 8{\cdot}61) = 6{\cdot}24$ sulphur in combination with calcium equivalent to 5·78 lime as hydroxyhydrosulphide and disulphide.

By calculation we obtain

	sulphur		lime
Hydroxyhydrosulphide	·365	associated with calcium equivalent to	·639
Disulphide	5·875	,, ,, ,,	5·141
	6·240		5·780

We therefore obtain the following results:

	sulphur		lime
Hydroxyhydrosulphide containing	·365	associated with calcium equivalent to	·639
Disulphide	5·875	,, ,, ,,	5·141
Free sulphur	8·61		
Thiosulphate	3·78	,, ,, ,,	3·31
Sulphate and sulphite ..	·09	, ,, ,,	·15
	18·72		9·24

[1] "The composition of some Lime-Sulphur Sprays made according to recognised formulæ," by A. A. Ramsay, read at Royal Society, N. S. Wales, 5.8.14.

The sulphur precipitated by the $\frac{N}{10}$ iodine solution in the determination of the so-called "monosulphide" sulphur was filtered off, dried, and weighed. The mean of four determinations gave 15·20. The figure which ought to have been obtained is ·365 plus 5·875 plus 8·61 = 14·85.

It is to be noted that this figure is again slightly too high, and by about the same amount, ·35, as was found in the analysis of mixture "A."

In the course of the investigation into so-called "polysulphide" sulphur, a method suggested by Tartar and Bradly[1] for the determination of "polysulphide" sulphur was tried. In this method $\frac{N}{10}$ hydrochloric acid is added to the sulphide solution till pink to methyl orange.

I find that this point corresponds closely to "monosulphide" determination by $\frac{N}{10}$ iodine or ammoniacal zinc chloride. I find also that titrating to the blue of congo-red gives a sharper end point.

$$CaS_x + 2HCl = CaCl_2 + H_2S + S_{(x-1)}.$$

The figures obtained were as follows:

	Calculated from $\frac{N}{10}$ iodine titration		Calculated from $\frac{N}{10}$ hydrochloric acid titration	
	sulphur	lime	sulphur	lime
Sample "A"	8·30	14·53	8·40	14·70
" "B"	6·88	12·04	6·96	12·18
" "C"	3·30	5·78	3·14	5·49

The close agreement between the two sets of figures in the cases of "A" and "B," where the quantity of thiosulphate is very small, *viz.* 0·99 and 1·62 respectively, and the greater divergence in the case of "C," where the quantity of thiosulphate is much larger, *viz.* 3·78, appears to indicate that the thiosulphate is the disturbing factor.

[1] "On the composition of Lime-Sulphur Sprays," by Tartar and Bradly. *Journal of Industrial and Engineering Chemistry*, II. p. 272.

SUMMARY.

In a previous paper[1] I have stated: "The monosulphide sulphur, therefore, appears to be essentially calcium hydroxyhydrosulphide CaHSOH with very minute quantities of calcium hydrosulphide CaH_2S_2. The solution of lime sulphur then appears to consist of calcium hydroxy-hydrosulphide, calcium thiosulphate, calcium sulphate with sulphur held in solution."

I have now to amend this by adding "calcium disulphide" to the above.

The method of analysis is now as follows: Preparation of sample. 50 c.c. of the concentrated lime sulphur to be diluted to 500 c.c. with water.

1. 25 c.c. of the diluted mixture is titrated with $\frac{N}{10}$ iodine solution till the yellow colour is discharged.

The number of c.c. used $\times \cdot 0016$ = sulphur (1) and the c.c. used $\times \cdot 0028$ = lime (1).

2. To the 25 c.c. diluted mixture used for determination 1, the addition of $\frac{N}{10}$ iodine solution is continued till a tinge of yellow colour obtains.

The number of c.c. $\frac{N}{10}$ iodine added $\times \cdot 0064$ = sulphur as thiosulphate and the number of c.c. $\frac{N}{10}$ iodine used $\times \cdot 0056$ = lime (CaO) in combination as thiosulphate.

Note. Starch paste or paper may be used in 1 and 2, but the addition of starch renders filtration of 2 difficult to proceed to 3.

3. The fluid used in 2 is filtered through double filter paper and washed with cold water. To the filtrate barium chloride and a few drops dilute hydrochloric acid are added, and the whole allowed to stand all night in the cold. Barium sulphate is filtered off, washed, dried, ignited and weighed. The weight obtained $\times \cdot 1373$ = sulphur present as sulphate and sulphite. The sulphur $\times$ 1·75 = lime equivalent to sulphur as sulphate and sulphite.

4. 10 c.c. of the diluted mixture is diluted with about 25 c.c. water, and is shaken up in a separating funnel with 10 c.c. carbon

[1] "The preparation and composition of Lime-Sulphur Sprays," by Ramsay, *Journal of Agricultural Science*, VI. pt. II. p. 201.

bisulphide, and allowed to separate. The carbon bisulphide is drawn off into a tared Erlenmeyer flask. The diluted mixture in the funnel is again extracted with 10 c.c. carbon bisulphide and finally with two lots of 5 c.c. each—the carbon bisulphide after extraction being added to that already in the tared flask. The carbon bisulphide is now removed by placing the flask in warm water. The flask and contents are now dried to constant weight at a low temperature (not exceeding 70° C.). The sulphur obtained is free sulphur.

5. To 10 c.c. of the diluted mixture, about 6 or 8 grams sodium peroxide is added to oxidise the mixture, which is allowed to stand a few minutes. 50 to 75 c.c. water is added, and then hydrochloric acid, cautiously, till the solution clears up. Add a few drops of potassium iodide solution (15 grams KI in 100 c.c. water) to reduce the higher oxides (of chlorine)—boil off the excess of iodine—dilute with water to about 200 c.c. and precipitate sulphur as $BaSO_4$. Filter, dry, ignite, weigh, and calculate to sulphur by multiplying weight of precipitate by ·1373. This gives total sulphur.

6. To another 10 c.c. aliquot of the diluted mixture, $\frac{N}{10}$ iodine solution is added as previously described till sulphides and thiosulphates are decomposed as at 1 and 2. Filter sulphur off through double filter paper. Make filtrate ammoniacal, and determine the lime by precipitation with ammonium oxalate. This gives total lime.

Calculations:

(*a*) Sulphur obtained at 5 minus [sulphur (4) plus sulphur (3) plus sulphur (2)] = sulphur as hydroxyhydrosulphide and disulphide = (*a*).

(*b*) Lime obtained at 6 minus (lime calculated at 3 plus lime calculated at 2) = lime as hydroxyhydrosulphide and disulphide = (*b*).

Let x = lime in combination as hydroxyhydrosulphide then $(b) - x$ = lime in combination as disulphide. Further $x \times \cdot 5714$ will be the sulphur in combination with x lime as hydroxyhydrosulphide and $(b) - x \times 1\cdot 1428$ the sulphur with $(b) - x$ lime as disulphide. Then

$$x \times \cdot 5714 + [(b) - x] \times 1\cdot 1428 = (a),$$

$$x = \frac{[(b) \times 1\cdot 1428] - a}{\cdot 5714},$$

from which $(b) - x$ is found.

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THE PROTECTIVE ACTION, AGAINST $MgCO_3$, OF $CaCO_3$ FOR *A. CHROOCOCCUM*.

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INTRODUCTORY.

SINCE 1892 when Loew first enunciated[1] his hypothesis in favour of the necessity of a certain ratio of lime to magnesia in the soil for the maintenance of proper conditions for plant growth, attempts have not been wanting on the part of many investigators in many places to adduce evidence from more or less extensive experiments which might reveal the facts of the situation; and to establish a basis for the treatment of soils if the hypothesis should prove correct. It is neither feasible nor desirable here to review the now rather voluminous literature on the hypothesis of the lime-magnesia ratio in its different bearings, especially since this is now being more completely done by one of us in a separate publication, and we therefore give below only a few statements which we deem pertinent in connection with that phase of the "lime magnesia" question herein dealt with.

Whatever may be the merits of Loew's theory, and particularly his claim to the validity thereof for practical purposes, no student of the literature of animal, plant, bacterial and fungal physiology can have failed to accept as valid and as of striking interest the more general conceptions of antagonism between ions, and of physiologically balanced solutions which we owe in their essence to Loeb. It may therefore be stated here that whether or not a general relation between lime and magnesia in soils is ever discovered which may serve the practical purposes of soil management, it is certain that ions both positive[2] and negative[3] can antagonize each other and through such antagonism render a certain measure of protection to the organisms living in the medium concerned.

[1] *Flora*, 1892, p. 368.

[2] *Ergeb. der Physiol.* **5**, 216.

[3] *Cent. fur Bakt.* 2te Ab. **36**, 382, and other work soon to appear in same Journal.

So far as the bacteria are concerned the researches dealing with the antagonism between ions have been confined, so far as we are aware, to those carried out by one of the writers or by one of the investigators associated with him. These investigations (all carried out with soil bacteria) and their results have been published in various papers which are cited or given in full in the publications referred to. The only investigations which are of direct interest here, however, are those[1] revealing the fact that for *B. subtilis* as an ammonifying organism no antagonism exists between calcium and magnesium or at least when the chlorides of those elements are employed; and those of Kelley[2] in this laboratory in which the natural flora of soil, in the latter as a medium, were studied with respect to the effect of $CaCO_3$ and $MgCO_3$ on the ammonifying and nitrifying bacteria therein existing. Not only was no antagonism found in the first work mentioned under the circumstances there obtaining but the solution in which the cultures were grown became more toxic when either of the chlorides was added to the other. Later investigations by the author of the paper just discussed proved, as above indicated, that the anion is of great significance in antagonistic effects as well as the kation. In view of that fact it might prove true that antagonism does exist between calcium and magnesium as regards the ammonifying power of *B. subtilis* if the proper compounds of those elements be employed. Whether this is so or not remains to be ascertained by future work, but until such is shown to be the case it must be assumed that important physiological differences with respect to the factor of ionic antagonisms obtain between *B. subtilis* and *A. chroococcum*, as is further shown in this paper. Kelley's investigations are even more directly comparable with those described below, since calcium and magnesium carbonates were employed and soil cultures used. Kelley could not obtain any toxic effects in using calcium carbonate in the cases of the two sandy soils studied by him, but did obtain a marked toxicity for the natural ammonifying and nitrifying flora of those soils with $MgCO_3$. He did not, however, observe any antagonism between calcium and magnesium carbonates for the bacteria mentioned, a result in partial harmony with that obtained by one of us as above described. In an unpublished communication to one of us Dr Kelley states that all soils do not behave as do the sandy soils mentioned with respect to $CaCO_3$ and $MgCO_3$. His experiments with the nitrogen fixing flora of the California soil were inconclusive and were not published.

[1] *Bot. Gaz.* **49**, 41. [2] *Univ. of Cal. Publ. Series in Agricultural Sciences*, **1**, 39.

A. *Solution Cultures.*

In the work with solutions we employed Ashby's mannite solution[1], which was distributed in 50 c.c. portions in 250 c.c. Erlenmeyer flasks. The solution, which in every case contained one gram of mannite, was made neutral to phenolphthalein with NaOH. The varying amounts of $CaCO_3$ and $MgCO_3$ were then added to the flasks of the two series respectively as indicated in the tables. The flasks were plugged, and then sterilized in the autoclave. When cool each was inoculated with 1 c.c. of a water suspension made from an agar slant culture of *A. chroococcum* derived from a slightly sandy soil from Anaheim, Cal. The cultures were incubated for three weeks at 28° to 30° C. At the end of that time they were analysed for total nitrogen in accordance with a modified form of the Gunning method described elsewhere[2] by one of us. The results are given for the effects of $CaCO_3$ and $MgCO_3$ in Tables I and II respectively which follow. The determinations were run in duplicate throughout.

It is evident from Tables I and II that calcium and magnesium carbonates are totally different from each other in their effects on *A. chroococcum*. The first is decidedly stimulating to the fixation of nitrogen by the organism under consideration in all concentrations, whereas the second is as decidedly toxic in all concentrations in excess of ·1 %.

While all concentrations of $CaCO_3$ are stimulating to *A. chroococcum* it must be noted, however, that there is not much more stimulation from the largest amounts than there is from the smallest amount of that compound. At any rate, the increased nitrogen fixation in cultures containing more than ·1 % $CaCO_3$ is too erratic in the different cultures and the discrepancies too large between duplicate cultures to justify one in concluding for example, as it appears superficially, that stimulation increases from ·1 % to and including ·4 % $CaCO_3$. It is certain, however, that no toxic effect is manifest in any of the cultures. The marked stimulation to nitrogen fixation induced by $CaCO_3$ is of course interesting but needs no further comment here since similar effects have been reported and explained in the past by several investigators. We presume that the equal effect of all quantities of $CaCO_3$ or what is virtually the same effect throughout is to be explained by the fact that

[1] *Jour. Agr. Science*, **2**, 35.
[2] *Jour. Biol. Chem.* **10**, 169.

·1 % of that salt is as effective in saturating the culture solution with respect to $CaCO_3$ as the larger amounts for reasons which appear obvious.

TABLE I. *The Effect of $CaCO_3$ on N. fixation by A. chroococcum in Mannite Solution.*

% $CaCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Avg. N. fixed mgs.	% $CaCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Avg. N. fixed mgs.
0	4·62	3·36	3·36	1·0	6·30	5·04	5·11
0	4·62	3·36		1·0	6·44	5·18	
·1	6·30	5·04	4·97	1·2	6·86	5·60	5·60
·1	6·16	4·90		1·2	6·86	5·60	
·2	6·86	5·60	5·53	1·4	6·72	5·46	5·11
·2	6·72	5·46		1·4	6·02	4·76	
·4	7·28	6·02	6·23	1·6	7·14	5·88	6·09
·4	7·70	6·44		1·6	7·56	6·30	
·6	6·02	4·76	4·76	1·8	6·72	5·46	5·25
·6	6·02	4·76		1·8	6·30	5·04	
·8	5·88	4·62	4·75	2·0	6·72	5·46	5·74
·8	6·16	4·90		2·0	7·28	6·02	

TABLE II. *The Effect of $MgCO_3$ on N. fixation by A. chroococcum in Mannite Solution.*

% $MgCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Avg. N. fixed mgs.	% $MgCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Avg. N. fixed mgs.
0	4·62	3·36	3·36	1·0	·98	−·28	−·21
0	4·62	3·36		1·0	1·12	−·14	
·1	5·60	4·34	3·57	1·2	2·80	1·54	1·26
·1	4·06	2·80		1·2	2·24	·98	
·2	·98	−·28	−·35	1·4	1·12	−·14	−·43
·2	·84	−·42		1·4	1·98	·72	
·4	·70	−·56	−·48	1·6	·98	−·28	−·28
·4	·84	−·42		1·6	·98	−·28	
·6	·84	−·42	−·35	1·8	·98	−·28	−·28
·6	·98	−·28		1·8	·98	−·28	
·8	1·12	−·14	−·28	2·0	·84	−·42	−·35
·8	·84	−·42		2·0	·98	−·28	

As above stated, however, the conditions are reversed with $MgCO_3$. This salt is acutely toxic at concentrations above ·1%, and even the latter concentration must be very near the toxic point since the duplicate cultures with that amount of $MgCO_3$ behave very erratically and vary markedly among themselves. One of them, as will be noted, shows a

slightly toxic effect of the $MgCO_3$, while the other shows a decidedly stimulating effect. Here, again, the explanation of the uniform toxicity of all amounts of $MgCO_3$ is probably to be referred back to a cause similar to that operating in the production of a uniform stimulation by all concentrations of $CaCO_3$, and, namely, the saturation of the culture solution with respect to $MgCO_3$. Since at ·2 % $MgCO_3$ as much magnesium goes into solution as in the higher concentrations and since further it is sufficient to inhibit nitrogen fixation by *A. chroococcum*, culture solutions are obtained which appear similar at all concentrations, being for purposes of nitrogen fixation sterile with one or two inexplicable exceptions like Nos. 7 and 8 in Table II.

There can be no doubt therefore that we are not dealing with materials that are similar in their physiological effects on *A. chroococcum*. It would appear that we are not confronted by toxic properties of both $CaCO_3$ and $MgCO_3$ when used in excess which Loew claims to be the case so far as plants are concerned. One of these compounds certainly is not toxic, whereas the other certainly is. So far as Azotobacter is concerned, therefore, there should never be any necessity for employing means to offset a possible toxicity of $CaCO_3$. There may well be such a need, however, in the case of $MgCO_3$ if the amount of $MgCO_3$ in the soil should prove to be large enough to be toxic to the soil bacteria.

So far as the use of $CaCO_3$ and $MgCO_3$, singly, is concerned, our results are entirely in harmony with those of Kelley first mentioned above even though ours were solution media with pure cultures of one organism while his were soil cultures in which the natural flora and different groups of organisms were studied. A closer comparison, however, will be drawn below in which the soil media in both cases may be compared.

Another question must be introduced into the discussion here which is of considerable interest and importance owing to the fact that it has figured in critical remarks made on some of the experimental work concerned with studies on the lime-magnesia ratio. The question referred to is the basicity of $MgCO_3$ as obtained on the market and its rôle in the toxicity of the compound. The latter is of course more caustic than $CaCO_3$ and the material employed in our experiments was a product of the Baker Chemical Company and answered to the formula $3MgCO_3 \cdot Mg(OH)_3 \cdot 3H_2O$. It was on this basis that Gile explained the toxic effects of $MgCO_3$ as manifested in experiments carried out by Hopkins[1] attributing the acutely poisonous effects (or

[1] *Soil Fertility and Permanent Agriculture*, p. 172. Ginn & Co., 1910.

of a large part thereof) to the caustic nature of the $MgCO_3$. In order to ascertain if this factor was to be considered of serious import in our experiments we determined by titration the alkalinity (methyl orange) of 50 c.c. of a saturated aqueous solution of the $MgCO_3$ employed, and then made up two Ashby solution cultures like those above described except that $\frac{N}{10}$ KOH was added in quantity sufficient to give a similar basicity to that of a saturated solution of the $MgCO_3$, no $CaCO_3$ or $MgCO_3$ being added, of course. The nitrogen fixed in these cultures otherwise treated like the other cultures was as follows:

	N. fixed mgs.
In 50 c.c. Ashby solution neutral to phenolphthalein	3·50
,, ,, ,, ,, ,, ,,	3·50
,, ,, ,, ,, ,, ,, $+ 2·1$ c.c. $\frac{N}{10}$ KOH	4·48
,, ,, ,, ,, ,, ,, + ,, ,, ,,	4·06

From the foregoing figures it is evident that the alkalinity due to the saturated solution of $MgCO_3$ (in the form used) not only is not toxic but is actually markedly beneficial. Gile's explanation therefore, so far as the higher plants are concerned whether true for them or not, is certainly not applicable to cultures of *A. chroococcum*.

B. *Soil Cultures.*

In this part of the experiment on the toxicity of $CaCO_3$ and $MgCO_3$ 50 gram portions of the Anaheim sandy soil above mentioned were distributed in tumblers, 1 gram of mannite and the necessary $CaCO_3$ or $MgCO_3$ added and stirred in the tumblers, covered with Petri dish covers, and sterilized in the autoclave for three hours at 25 lbs. pressure. When cool the soil in each tumbler was inoculated with a 1 c.c. suspension of an *A. chroococcum* culture prepared as above described. The necessary sterile distilled water was then added to make optimum moisture conditions and the soil thoroughly stirred with a sterile spatula. These soil cultures were then incubated for four weeks at 28° to 30° C., at the end of which period the soils were dried at 100° C. and ground, and 20 gram portions taken for analysis for total nitrogen in accordance with the method used above in the case of the solution cultures. The other data with reference to the details of the experiment and the results of the nitrogen determinations, showing the amounts of nitrogen fixed in the various cultures, are given for $CaCO_3$ and $MgCO_3$ respectively in Tables III and IV.

TABLE III.

The Effect of $CaCO_3$ on N. fixation by A. chroococcum in Soils.

% $CaCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Avg. N. fixed mgs.	% $CaCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Avg. N. fixed mgs.
0	30·10	7·70	6·30	1·0	28·00	5·60	7·00
0	27·30	4·90		1·0	30·80	8·40	
·1	28·00	5·60	5·95	1·2	30·10	7·70	6·65
·1	28·70	6·30		1·2	28·00	5·60	
·2	26·60	4·20	5·25	1·4	25·90	3·50	2·80
·2	27·30	6·30		1·4	24·51	2·10	
·4	26·60	4·20	4·55	1·6	23·80	1·40	1·75
·4	27·30	4·90		1·6	24·50	2·10	
·6	29·40	7·00	5·60	1·8	23·10	·70	1·75
·6	26·60	4·20		1·8	25·20	2·80	
·8	28·00	5·60	4·55	2·0	23·10	·70	2·10
·8	25·90	3·50		2·0	25·90	3·50	

TABLE IV.

The Effect of $MgCO_3$ on N. fixation by A. chroococcum in Soils.

% $MgCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Avg. N. fixed mgs.	% $MgCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Avg. N. fixed mgs.
0	30·10	7·70	6·30	1·0	23·80	1·40	3·15
0	27·30	4·90		1·0	27·30	4·90	
·1	24·50	2·10	1·75	1·2	21·70	– ·70	·35
·1	23·80	1·40		1·2	23·80	1·40	
·2	23·80	1·40	1·40	1·4	22·40	0	·35
·2	—	—		1·4	23·10	·70	
·4	25·20	2·80	1·75	1·6	22·40	0	1·05
·4	23·10	·70		1·6	24·50	2·10	
·6	22·40	·0	1·05	1·8	25·20	2·80	2·80
·6	24·50	2·10		1·8	—	—	
·8	27·30	4·90	3·85	2·0	23·80	1·40	·70
·8	25·20	2·80		2·0	22·40	0	

The data in Tables III and IV indicate plainly that the effects of $CaCO_3$ and $MgCO_3$ on the nitrogen fixing power of *A. chroococcum* are very much the same in the soil as a medium as they are in mannite solutions, in a general way. That is to say, $CaCO_3$ may for all practical purposes be considered non-toxic and $MgCO_3$ very toxic. Two noteworthy differences, however, obtain between the action of $CaCO_3$ in soils and in solutions. It manifests a slightly toxic effect in soils when it is

added in concentrations in excess of 1·4 % of the dry weight of the soil; it shows no toxic effects in solutions when used in concentrations of 2 % and even gives a marked stimulation. $MgCO_3$ behaves in a slightly more toxic manner in soils than in solutions, the smallest concentration markedly inhibiting nitrogen fixation. Otherwise it behaves in all essential respects in soils like it does in solutions.

In the publications, above mentioned, by Kelley a brief statement is made anent experiments carried out with the natural nitrogen fixing flora of the Anaheim soil as regards the effect of $CaCO_3$ and $MgCO_3$. Kelley claims that the results obtained were "irregular and discordant." In one series a slight toxic effect of $MgCO_3$ was observed, while in another series no effect was observed. Our experiments, which only differed from Kelley's, so far as we are aware, in the fact that we worked with pure cultures obtained from the same soil whereas he worked with the natural flora, yielded very definite data and we believe justify the conclusions above drawn. Again comparing the results in Tables III and IV with those obtained by Kelley with the ammonifying flora of the Oakley sand and the nitrifying flora of the Anaheim sand it appears that $MgCO_3$ affects *A. chroococcum* in soil very much as it does the other organisms just mentioned in their respective soils.

On the other hand, $CaCO_3$ was found by Kelley to be slightly stimulating to ammonification and strikingly so to nitrification, but we do not find it to be a stimulant to nitrogen fixation by *A. chroococcum* in soils. What is even more interesting in this connection is the fact that our own results in solutions and in soils with the same organism are wholly at variance. In the one case marked stimulation is obtained but in the other case none. This forms another link in the chain of evidence which we have been building up against the attempt to apply data, obtained in solution culture work whether with plants or soil bacteria, to field soils without reservation. We make this assertion with a full knowledge of the discrepancies which exist even among different soils in these respects which make it unjustifiable to assume that any data obtained with one soil are wholly or sometimes even in part applicable to another soil. In this connection it may be added here that in a personal communication to one of us recently Kelley makes the statement that in the case of Hawaiian soils with which he has worked, since the experiments, above attributed to him, were completed, he finds results at variance with his earlier ones. For example, $MgCO_3$ is generally stimulating to ammonification in the Hawaiian soils studied, while it is toxic to the ammonifying flora of the Oakley soil. Still it seems to

be *generally* toxic to the nitrifying flora of those same soils. In some instances it was not toxic even to the nitrifying bacteria up to a concentration of 2 %. On the other hand, Kelley finds that in these same soils $CaCO_3$ is much less stimulating to ammonification than $MgCO_3$ and only slightly stimulating to nitrification.

In brief, and summarizing the results obtained with $CaCO_3$ and $MgCO_3$ each used singly, in soils and in solution, it would appear to be true first that $CaCO_3$ differs in its effects on *A. chroococcum* depending on the nature of the culture medium, but that it is either innocuous or stimulating, manifesting only in very large quantities and only in the soil as a medium a slight toxicity. Secondly, $MgCO_3$ is beyond any cavil acutely toxic in both solution and soil media to *A. chroococcum*, and is so because of the toxic nature of the Mg-ion and not because of the causticity of the carbonate as obtained in the chemically pure form.

The Antagonism between Calcium and Magnesium for A. chroococcum.

A. *Solution Cultures.*

The solution cultures in the antagonism series were prepared in the same way as in the series in which the salts were used separately with the exception that $CaCO_3$ was mixed in varying proportions with a constant toxic quantity of $MgCO_3$ as indicated in the tables. Also the incubation was carried out at room temperature for six weeks. All other explanatory data are to be found in Table V which follows.

It will be noted first that a constant toxic quantity of $MgCO_3$ (·2 %) was employed throughout. This it will be remembered was sufficient to inhibit nitrogen fixation by *A. chroococcum*, as reference to Table II will show. As a result of its high toxicity the bacteria did not develop satisfactorily in the incubator (even when $CaCO_3$ was added) where they were placed for three or four days at first. We therefore placed them in a dark closet at room temperature, or slightly below, for a period of six weeks. Under these conditions it will be noted that *A. chroococcum* could develop and even fix appreciable quantities of nitrogen despite the presence of ·2 % $MgCO_3$. The reduced toxicity of the latter in this series is doubtless to be attributed to its much smaller solubility at the lower temperature and possibly also to a gradual adaptation of *A. chroococcum* to the effects of the salt during the longer period of incubation.

TABLE V. *The Antagonistic effect of $CaCO_3$ to the toxicity of $MgCO_3$ as measured by N. fixation by A. chroococcum in Mannite Solution.*

% $MgCO_3$ added	% $CaCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Av. N. fixed mgs.
0	0	6·72	5·46	5·39
0	0	6·58	5·32	
·2	0	3·36	2·10	2·03
·2	0	3·22	1·96	
·2	·25	3·22	1·96	1·75
·2	·25	2·80	1·54	
·2	·50	3·50	2·24	2·17
·2	·50	3·36	2·10	
·2	·75	3·78	2·52	2·31
·2	·75	3·36	2·10	
·2	1·00	4·34	3·08	2·80
·2	1·00	3·78	2·52	
·2	1·25	4·48	3·22	3·50
·2	1·25	5·04	3·78	
·2	1·50	4·20	2·94	3·01
·2	1·50	4·34	3·08	

Turning now to a discussion of the salient features of Table V, we find first, that $CaCO_3$ has the power of antagonizing the toxic effects of $MgCO_3$ and its effects begin to show only when ·75 % $CaCO_3$ or more is added to ·2 % $MgCO_3$, the maximum antagonistic effect being apparently attained by a combination of ·2 % $MgCO_3$ and 1·25 % $CaCO_3$. Larger additions of the latter salt do not seem to be as favourable. This is one of those cases of antagonism in which a stimulating or non-effective salt antagonizes a toxic salt. One of the writers has gone into a discussion of the theoretical phases of this question elsewhere[1] and begs to refer the reader to that publication if he should be interested. Even, however, if we should disallow the use of the term "antagonism" in cases in which all salts employed are not toxic we are compelled at least to recognize the "protective" effect in the present work of $CaCO_3$ for *A. chroococcum* against $MgCO_3$ when solution cultures are employed. Fuller discussion and comparison with other work is given in the next section on analogous work in soil cultures.

[1] *Cent. für Bakt.* 2te Abt., **36**, 383.

B. *Soil Cultures.*

The preparation of the cultures in this part of the experiment was again the same as in the case of the preceding soil cultures described in Tables III and IV except that $MgCO_3$ was taken in constant toxic quantity throughout (·1 %) and $CaCO_3$ added in varying quantities as shown in Table IV. Unlike the procedure of the series with solution cultures just preceding, the incubation with the present soil cultures was carried out in the incubator at the same temperature and for the same length of time as the other soil cultures. The results obtained follow in Table VI.

TABLE VI. *The Antagonistic effect of $CaCO_3$ to the toxicity of $MgCO_3$ as measured by N. fixation by A. chroococcum in Soils.*

% $MgCO_3$ added	% $CaCO_3$ added	N. found mgs.	N. fixed per gram mannite mgs.	Av. N. fixed mgs.
0	0	32·20	4·98	5·16
0	0	32·55	5·33	
·1	0	30·45	3·23	3·41
·1	0	30·80	3·58	
·1	·25	30·10	2·88	3·76
·1	·25	31·85	4·63	
·1	·50	32·90	5·68	5·33
·1	·50	32·20	4·98	
·1	·75	31·85	4·63	4·46
·1	·75	31·50	4·28	
·1	1·00	33·25	6·03	5·68
·1	1·00	32·55	5·33	
·1	1·25	32·90	5·68	5·68
·1	1·25	32·90	5·68	
·1	1·50	33·95	6·73	6·90
·1	1·50	34·30	7·08	

Even a casual examination of the foregoing table will make it quite clear that $CaCO_3$ has the power of antagonizing $MgCO_3$ or at least of exercising a "protective" action for *A. chroococcum* against $MgCO_3$. So far as such antagonism is concerned also we find greater harmony of action between the solution and soil cultures than when the salts are used separately. It appears, however, that in the soil cultures much smaller quantities exercise antagonistic effects than in the solution cultures.

Referring our results again to those of Kelley above cited, it would appear that *A. chroococcum* behaves in an entirely different manner from the ammonifying flora of the Oakley soil or from that of the

nitrifying flora of the Anaheim soil. As shown in the paper by Kelley above referred to, no antagonism between calcium and magnesium could be noted in the latter two cases, but we have obtained antagonism for *A. chroococcum* as shown in Tables V and VI in both mannite solutions and in soils. While these antagonisms show differences in different media they are differences in degree and not in kind. It should be added also that in the personal communication, received by one of the writers from Kelley, above referred to, the most striking statement was that no matter how $CaCO_3$ and $MgCO_3$ varied in their action under different circumstances so far as ammonifying and nitrifying flora of Hawaiian soils are concerned they acted throughout uniformly with respect to antagonism and consistently showed none whatever. This is again a result entirely at variance with ours and indicates either that *A. chroococcum* is differently affected in its functions than other soil bacteria by certain chemicals or that different soil media so vitally affect the activation of the bacterial cell and the nature of their natural flora as to render it questionable if results obtained with soil bacteria in one soil possess any cogency whatever for other soils.

Summarizing the data obtained in the antagonism experiments with $CaCO_3$ and $MgCO_3$ in both solutions and in soils, we feel compelled to conclude that $CaCO_3$ has a protective action for *A. chroococcum* against the toxic effects of $MgCO_3$; that such protective action occurs in solution cultures as well as in soil cultures; that the degree of protection varies with the amount of $CaCO_3$ in different media from a slight one to a very marked one; and finally that *A. chroococcum* is the only soil organism with which it has thus far been possible to obtain indubitable evidence of antagonism between $CaCO_3$ and $MgCO_3$, the ammonifying and nitrifying flora having been shown by Kelley to be unaffected by the lime-magnesia ratio or by any protective action such as that under discussion here.

Discussion.

Loew has attempted[1] to explain the lack of antagonism between $CaCl_2$ and $MgCl_2$ as demonstrated by one of the writers in his work with *B. subtilis* above cited by making the assertion that bacteria and that type of lower plant life do not need calcium for their life processes, or in other words, that that element is not essential for them as it is for the chlorophyll bearing plants. How then are we to explain the existence of antagonism between the calcium and magnesium demonstrated in this paper for another form of soil bacteria—*A. chroococcum*?

[1] *Bot. Gaz.*, **49**, 304.

The writers are of course cognizant of the fact that the Azotobacter organisms are "lime loving" organisms and very much more closely related to chlorophyll bearing organisms than is *B. subtilis*, or the group the latter represents, nevertheless they certainly are devoid of chlorophyll as such and should therefore be expected to behave like other soil bacteria and not like the higher plants. We do not feel that Loew's explanation is at all satisfying nor adequate for the reason just given. While we frankly admit that the varying experimental evidence in hand as cited does not give us leave to put forward any further theories, it would likewise appear that much more work must be carried on before the question of the relations between lime and magnesia in soils in its broader phases and as regards both plants and soil bacteria may be fully understood.

So far as the investigation under immediate discussion is concerned, however, we feel that there can be no doubt that we have demonstrated several important facts with reference to the relationships between calcium and magnesium carbonates and *A. chroococcum*. We have shown, for example, that physiologically speaking $MgCO_3$ is totally different from $CaCO_3$ and that it is very toxic in both soil and solution cultures, whereas the last named salt is not. In this connection, it may perhaps be questioned if the slight toxicity manifested by $CaCO_3$ in soil cultures is not of more serious significance than our statements above would lead one to believe. It may be argued, for example, that a slight toxicity of $CaCO_3$ at a concentration of 1·5 % of the dry weight of the soil may mean in practice that our distinctly calcareous soils would be unsuited to proper Azotobacter development. It must be remembered, however, that while 1·5 % of $CaCO_3$ is equivalent to a lime content not infrequently met with in soils, for example of the arid region, the soil which contains that quantity of lime in the carbonate form, in its upper layers is rather exceptional than otherwise. It may be added also that the soil medium itself before the calcium carbonate was added already carried a total lime content in excess of 1 %, thus making the toxic point for $CaCO_3$ really a very much higher one than that which seems apparent from the data above submitted.

Then, the other points demonstrated above are the innocuous or even stimulating nature for *A. chroococcum*, of the alkalinity of $MgCO_3$ and the most important point of the investigation, namely the demonstration of the protective service rendered by $CaCO_3$ for *A. chroococcum* against the toxic effects of $MgCO_3$. Both of these points are invested not only with a measure of scientific interest but also with practical

significance. Their exposition would appear to make it probable that in field practice the use of $MgCO_3$ as far as its causticity is concerned is not to be considered dangerous to the Azotobacter organisms of soils, and that in soils in which an excess of magnesium may be found, so far as Azotobacter is concerned, the harmful effects of such an excess may be offset by $CaCO_3$ applications.

Lastly, we desire to add that this whole question is as complicated from the soil-bacteriological standpoint as it is from that of plant physiology. Especially when we employ the soil as a medium, and one cannot well see how we can escape that if we are to obtain results of practical value, we deal not only with the relationships of calcium and magnesium alone, but as Gile[1] has so aptly pointed out, also with the relationships of calcium and magnesium to all other constituents of the soil solution. Of these there are of course many, and in widely varying amounts, to say nothing of the enormous qualitative differences which must obtain between the chemical composition of solutions from various soils. We believe that this very much involved problem in all its complications can only be solved through patient effort applied to one factor at a time and that in both solutions and in soils of great variety. While such a programme may seem impossible of accomplishment, mature thought will lead one to the conclusion that it is not and, moreover, that it must be carried out if we are to make the much desired and very desirable progress in the solution of the intricate and intensely complicated problems of plants and soils which confront us.

SUMMARY.

In investigations on the effects of calcium carbonate and magnesium carbonate on nitrogen fixation by *A. chroococcum* in soils and in solutions, the following results have been obtained:

1. Calcium carbonate in mannite solution cultures acts only as a stimulant never as a toxic agent at least up to and including a concentration of 2 %.

2. Magnesium carbonate is sharply toxic in solution cultures in concentrations in excess of ·1 % to ·2 %.

3. Calcium carbonate in soil cultures is without effect up to 1·4 % of the dry weight of the soil when it becomes slightly toxic in the Anaheim sandy soil.

[1] Bul. No. 12, Porto Rico Agr. Expt. Station.

4. Magnesium carbonate is even more toxic in soil cultures than in solution cultures when the Anaheim sandy soil is used. ·1 % magnesium carbonate is sufficient to inhibit nitrogen fixation almost totally.

5. The causticity of chemically pure magnesium carbonate as prepared by the Baker Chemical Company does not account for the toxic effect of magnesium carbonate. The latter effect must be due to the magnesium ion. The alkalinity of magnesium carbonate is beneficial, rather than otherwise, to *A. chroococcum.*

6. Calcium carbonate exercises a protective action in solution cultures for *A. chroococcum* against the toxic properties of magnesium carbonate. The best ratio seems to be six to one ($CaCO_3 : MgCO_3$) when the absolute values are ·2 % $MgCO_3$ and 1·25 % $CaCO_3$.

7. Calcium carbonate exercises a protective action in soil cultures for *A. chroococcum* against the toxic properties of magnesium carbonate. The best ratio seems to be 15 : 1 ($CaCO_3 : MgCO_3$) when the absolute values are ·1 % $MgCO_3$ and 1·50 % $CaCO_3$.

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PASTURE PROBLEMS: THE RESPONSE OF INDIVIDUAL SPECIES UNDER MANURES.

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I. Introduction.

Manurial experiments on grassland have been carried out on permanent plots at Rothamsted[1] since 1856, at Cirencester[7, 8] since 1888, at Cockle Park[9] since 1897; and in recent years at a number of other stations. The effect of manurial treatments has been gauged on meadows by comparing the weight of the produce from the several plots, on pastures by comparing the live-weight increase of stock, or by comparing the milk yield of cattle fed upon the plots. Analyses have been made at several stations to show the action of manures on the botanical composition of the herbage on plots variously treated. Results given by all these methods are purely empirical.

It is proposed in this article to examine in detail the behaviour of a few typical species under the action of manures, and furthermore to study the action of these species in relation to the types of grassland on which definite experiments have been carried out. It will be shown in conclusion that the competitive interaction of species under manures is governed by certain guiding principles.

II. Types of Grassland.

It is now generally recognised that the old natural and semi-natural grasslands of these Islands are capable of being classified according to fairly definite botanical characteristics. It is found furthermore that the various types are associated with definite geological formations or topographical features. Smith and Crampton[3] have traced some of the main features of British grassland. Armstrong[2] has worked out the botanical composition of some of the best feeding pastures of the

country. The present writer, when at Cirencester, made a study of the chief types of grassland on the Cotswolds (4), (6), (7), and now at Aberystwyth is engaged upon a survey of the Sheep Walks of Mid-Wales (5).

Any particular type of grassland is to be regarded as a plant community[1], which maintains a more or less constant physiognomy—a physiognomy which remains constant both on account of the botanical composition of the herbage and on account of the manner of growth of the contributing species. As long as the environment remains unchanged the community will remain unchanged. The prevailing husbandry may fairly be regarded as an environmental factor, which husbandry in the case of sheep grazing, cattle fattening, and often, also, in regard to the periodic taking of hay, is no less constant than the meteorological factors of environment in this country.

The preliminary recognition of true types is a matter of experience and local knowledge; but by repeated botanical analyses it is possible to establish a correct formula for each true type. This is done by making as many analyses as possible on a given type[2]. The results are tabulated to show the percentage contribution of each species to the total herbage. These figures will show which are the chief contributing species which, in fact, are the *fundamental* species. For these species the analyses furthermore establish three things:

(1) The most usual (taken as the average) contribution of a particular species, this may be called the *optimum* figure for that species.

(2) The largest contribution of a particular species, this may be called the *maximum* figure for that species.

(3) The smallest contribution of a particular species, this may be called the *minimum* figure for that species.

These figures may be called the cardinal figures for each species; they will be exact in proportion to the number of analyses made. They give, however, a very fair index of the limitations of each species within a definite plant community.

A "type" may now be defined in terms of its cardinal figures as: a plant community consisting of fundamental and subsidiary species, and being such that the contribution of each fundamental species to the total flora tends to be close to an *optimum* figure and does not increase above a certain *maximum* or decrease below a certain *minimum*.

Thus, for well-established types of grassland, the range between

[1] The word community is advisedly used, since at present no attempt has been made to give these grassland types a definite ecological status.

[2] For methods of analyses see (2) and (4).

a plant's minimum and maximum of distribution represents that amount of fluctuation which synchronizes with relatively small seasonal and adventitious environmental change. Great change in environment causes even the chief species either to advance beyond their maxima or to recede below their minima, that is to say fundamentally alters the character of the community, and so gives rise to a different type.

Four types of grassland will here be considered. They have been selected because the necessary botanical analyses are available and because manurial trials have been conducted on each of them.

The subjoined table (Table I) gives the cardinal figures for the fundamental species contributing to these types:

TABLE I. *To show the cardinal figures for the fundamental species on four types of grassland.*

(All the figures are derived from analyses made by the present writer.)

(1) Calcareous soils over Great Oolite: Cirencester (from 8 analyses).

Altitude 400′	Minimum	Optimum	Maximum
Bromus erectus	41	50	68
Festuca ovina	4	14	17
Dactylis glomerata	3	11	21
Lolium perenne	Trace	4	7
Trifolium repens	1	3	8
Weeds	2	7	10

(2) Residual Clay over Oolite: Cirencester (from 8 analyses).

Altitude 400′	Minimum	Optimum	Maximum
Lolium perenne	2	21	40
Dactylis glomerata	4	15	32
Festuca ovina	2	13	27
Avena flavescens	2	6	13
Trifolium repens	1	7	13
Weeds	2	8	16

(3) Good second class pastures Ordovician Shales: Aberystwyth (from 6 analyses).

Altitude 400′	Minimum	Optimum	Maximum
Dactylis glomerata	4	20	50
Cynosurus cristatus ..	1	10	19
Lolium perenne	1	3	6
Agrostis vulgaris	2	10	24
Trifolium repens	9	21	29
Weeds	7	13	25

(4) Heath: Fescue-Agrostis Pastures: Welsh Sheep Walks (from 10 analyses).

Altitude 600′–900′	Minimum	Optimum	Maximum
Festuca ovina	10	35	54
Agrostis vulgaris et alba ..	5	15	50
Holcus lanatus	1	3	15
Triodia decumbens ..	1	8	20
Trifolium repens	2	4	8
Weeds	7	15	30

III. Comparison of the Behaviour of Species under Manures at Different Stations.

The results of the manurial trials carried out on each of the selected types are summarized in Table II. On residual clays over Oolite two sets of manurial experiments are given, on the other types results from only one trial are available.

It will be convenient to study the behaviour of each of the species mentioned in the above tables in turn. In compiling the following notes, the results from other stations have also been drawn upon for the sake of comparison[1].

(The figures in brackets after each of the following species denote its greatest range of increase + and decrease − above and below the untreated plots.)

Agrostis or Bent: (*Agrostis stolonifera et A. vulgaris*) (− 52 to + 12).

On the average dressing with ammonium sulphate tends to produce considerable increase. When, however, this grass is very abundant (*i.e.* near its maximum figure) the increase is usually very small; when unusually abundant (*i.e.* probably above its maximum figure) such dressings may actually decrease it, this has occurred on one occasion at Cockle Park. Sodium nitrate generally decreases it, the same is usually true of the phosphatic manures. When, however, the plant stands at its minimum figure or is only a subsidiary component of the herbage, its reaction to all manures appears to be but slight and irregular. When standing between its minimum and optimum figures, decrease is usually slight, and increases may be occasioned by such a manure as sodium nitrate (*e.g.* at University Farm, Aberystwyth).

When near its maximum figure great decreases are usual, for instance at Glandyfi, where, standing at its maximum, it is decreased by 39 % by superphosphate.

Standing at high figures, it has been reduced by 30% by basic slag at Cockle Park, by over 30% by ground limestone at Lancaster, and by 52% by organic nitrogenous manures at Garforth.

Sheeps Fescue. (*Festuca ovina et vars.*) (− 7 to + 40).

This grass which usually has a wide range between its *minimum* and *maximum* figures on all pasture types shows a correspondingly

[1] Published results drawn upon in compiling the tables are Cirencester (7) and (8). Results from other stations are quoted from Rothamsted (1), Cockle Park (9), University of Leeds Manor Farm, Garforth (10), Lancaster Agricultural Dept. (11). The Aberystwyth results are drawn from the author's unpublished notes.

TABLE II. *To compare the results of different manurial treatments on several species, at different stations.*

(− indicates a decrease below, + a rise above, and 0 no change from the percentage on untreated plot.)

Species	A = Plots on pasture type 1				Plots on pasture type 2 B			Plots on pasture type 2 C			D = Plots on pasture type 3			E = Plots on pasture type 4		
	Cirencester Chapel Close				Dry Leaze Cirencester			Tarlton Cirencester			Univ. Farm Aberystwyth			Glandyfi Aberystwyth		
Agrostis stolonifera et A. vulgaris	—	—	—	—	3·5	− 2	+ 1·1	·2	0	+ ·1	6	− 2·4	+ 9	49	− 39	− 27
Festuca ovina et vars.	4·4	+ 2·9	+ 1·8	+ 30	24·6	− 1·3	− 1	6·2	+ 8	+ 2	—	—	—	18	+ 20	+ 18
Dactylis glomerata	3·2	− 7·6	+ 2·1	+ 7	4·1	+ 4·9	+ 3·2	18·5	+ 2·6	− 1·1	50·4	− 6·2	− ·1	—	—	—
Lolium perenne	·2	+ 4·1	+ 5·9	+ 9·2	10·6	+ 13·2	− ·6	29·3	− 1	− 13·2	—	—	—	—	—	—
Bromus erectus	57·2	+ 6·8	− 1·2	− 26·4	—	—	—	—	—	—	—	—	—	—	—	—
Trifolium repens	6·6	− 4·2	+ 4·6	+ 4	13·4	− 2·4	+ 17·5	·5	− ·9	+ 3·8	9	+ 6·7	− 3·5	4	+ 10	+ 12
Miscellaneous plants	2·4	− 2·2	− 1	+ 2·4	7·2	− 2·4	− ·1	13·1	− 10	− 5·4	11·7	− 7·3	− 6·7	17	0	0
Treatment	Unmanured	Sulphate of ammonia superphosphate and kainit	Superphosphate and kainit	Organic nitrogenous manures	Unmanured	Sulphate of ammonia superphosphate and kainit	Superphosphate and kainit	Unmanured	Sulphate of ammonia superphosphate and kainit	Superphosphate and kainit	Unmanured	Superphosphate	Potash and nitrate	Unmanured	Superphosphate	Superphosphate and potash

wide plasticity under manures. Its response is also very dependent on soil conditions. On the calcareous soils at Cirencester, farmyard manure causes the greatest increase, on boulder clay at Cockle Park lime has a great effect.

At Rothamsted ammonium sulphate produces great increase and sodium nitrate considerable decrease, while at Cirencester both these manures produce increase.

The behaviour of this grass is, however, most dependent on its degree of abundance on the plots. At Cirencester, standing at its minimum figure, it has been increased (on 18 plots) by all manurial dressings, except kainit alone. At Glandyfi and Tarlton, standing between its minimum and optimum figures, it has been increased. At Dry Leaze, standing at its maximum figure, it has been slightly decreased by dressings which at Tarlton produced increase.

Cocksfoot. (*Dactylis glomerata*) (– 6 to + 30).

Much more prone to increase than decrease; at some stations sodium nitrate and at others ammonium sulphate have proved most successful. Its degree of reaction is considerably modified by its abundance on the plots.

It is capable of great increase even when present in insignificant quantity (*e.g.* Rothamsted and Cockle Park). At its minimum figure it is invariably increased (*e.g.* Cirencester and Dry Leaze). At about its optimum figure (*e.g.* Tarlton) it has not much changed. At its maximum figure (*e.g.* Aberystwyth) it has been decreased. Under continued dressings with complete minerals and ammonium salts at Rothamsted it shows an interesting relation to its cardinal figures. During 1856–1862, it has risen from about 1 %–2 % to 24 % (presumably to something between its optimum and maximum figures), by 1867 it has further risen to 39 % (presumably its maximum figure), in 1872 it still stands at 39 % (its maximum), by 1877 it has fallen to 17 %, and by 1903 it has fallen to 5 % at which it has remained constant.

Perennial Rye Grass. (*Lolium perenne*) (– 13 to + 13).

Nitrogenous manures usually increase it, at some centres nitrate and at others ammonium sulphate doing best. Considerable increases are possible under these manures even when the grass stands at a low minimum (*e.g.* Cirencester); at Dry Leaze at a figure midway between its minimum and optimum it is increased to just over its optimum. At Tarlton, standing at about its optimum figure, it is unaltered. Phosphatic manures appear at some centres to considerably increase it, at others to greatly decrease it.

Thus at Tarlton where the grass stands well above its optimum a considerable decrease occurs, at Dry Leaze, where it stands below its optimum, a slight decrease occurs, whilst at Cirencester, where it stands at its minimum, an appreciable increase shows itself. The Tarlton [8] plots give a further clue to this irregular action under phosphates. We there have the following relationships.

Unmanured Plot:

Total *Leguminosae* : Rye Grass : : 10 : 29.

Superphosphate and kainit:

Total *Leguminosae* : Rye Grass : : + 21 : − 13.

Basic Slag:

Total *Leguminosae* : Rye Grass : : + 10 : + 3.

This shows that where very considerable increase takes place in the leguminosae, and where rye grass is also plentiful (between its optimum and maximum figures), the increase of the former is correlated with a decrease in the latter. Here the poorer action of basic slag even allows of a slight increase in the rye grass. At Cirencester, however, where rye grass increases considerably under phosphatic manures, it is not in such close competition with the "clovers," since it is only on the field to its minimum amount, nor are the "clovers" so plentiful and do not increase to such a high figure as at Tarlton. The uncertain behaviour of this grass under phosphates seems therefore to be governed by its competitive interaction with the clovers rather than by subtle differences in soil.

Upright Brome. (*Bromus erectus*) (− 37 to + 21).

This typical Cotswold grass, standing rather above its optimum, shows a tendency to be increased by kainit alone, and by dressings with kainit; it is very considerably decreased by dressing with farmyard manure or guano.

Dutch Clover. (*Trifolium repens*) (− 7 to + 16) is invariably increased by dressings with phosphates.

This reaction may be very considerable even when the plant stands at its minimum figure (*e.g.* Aberystwyth), or when present in the merest trace (*e.g.* Tarlton); it is also capable of considerable increase above its maximum figure (*e.g.* Dry Leaze and Glandyfi). Similarly it tends, irrespective of its abundance on the plots, to be decreased by inorganic nitrogenous dressings.

Miscellaneous Plants (– 24 to + 20).

Weeds are not always decreased by manuring, for organic nitrogenous manures have a decided tendency to increase them. Basic slag may greatly increase daisies. Ammonium sulphate has, however, a decidedly depressing influence on weeds.

The following grasses, although not fundamental species in the communities here under review, deserve mention.

Yorkshire Fog. (*Holcus lanatus*) (– 7 to + 40).

Is peculiarly fickle under manures. This is probably due (1) to the fact that it is very sensitive to the available water of the habitat (see [12]) and (2) that it fruits very freely, drops its seed early, and consequently increases considerably after dry seasons; facts which tend to mask its behaviour under manures. No manurial treatment seems certain to decrease it; lime often acts in this direction, and at Rothamsted continued applications of ammonium sulphate alone have done so. At most centres ammonium sulphate with mineral dressings causes considerable increase.

Meadow Grasses. (*Poa pratensis* and *Poa trivialis*.)

These grasses are very plastic under variations of the water-content of the habitat, dry conditions favouring the former and wet the latter. The former, however, tends to increase under dressing with ammonium salts, and the latter under those with nitrate.

IV. Summary and Conclusions.

The foregoing facts clearly show that the reaction of species to manures is not a simple function of the chemical influences of particular substances on the growth of individual plants. They prove, however, that the final adjustment reached by the various plants is profoundly affected as the result of competitive interaction between all the species which contribute to the herbage. Furthermore, it has been shown that if manurial plots are studied in the light of the type of grassland on which the experiments are carried out, that the influence of this competitive interaction can be fairly accurately gauged. The principles which regulate the resorting of species under the influences of a disturbed equilibrium are, broadly stated, as follows: if the changes in environment are not of a fundamental character, the tendency will be for plants near their maximum distribution to be decreased, for those near their minimum to be increased, and for those near their optimum to be

increased or decreased within the limits of their highest and lowest cardinal figures.

If the changes in environment are of a fundamental character, some of the species will tend to exceed their maximum and others to pass below their minimum figures; that is to say type alteration will occur. When the equilibrium is disturbed under the influence of manures, it will thus be seen that the behaviour of species in relation to their cardinal figures gives a valuable index as to the intensity of action of any particular treatment.

It must be remembered that a grassland type is a complex community of a number of different species, representing a number of different growth forms and physiological relationships towards the habitat. Thus any circumstance which disturbs the edaphic or aerial factors of habitat will, in the first instance, favour the growth (and therefore the spread) of some species more than of others; and will consequently, to some extent, re-awaken interspecific competition, or, at all events, alter the trend, and probably increase the intensity, of its action.

It has been noticed that plants endowed with a creeping habit are very plastic under the influences of altered climate[1] and of added manures; and have a wide range between their cardinal figures on most pasture types. It is at present impossible to give physiological reasons for the behaviour of plants (irrespective of their morphological characters).

In order to further elucidate the botanical results given by manurial trials, it will be advantageous to briefly review the aetiology of the action of manures.

THE AETIOLOGY OF THE ACTION OF MANURES.

The final re-adjustment of the species contributing to the herbage of any plot will be influenced by the operation of the following factors.

A. *Factors which are always operative; and which will tend to exaggerate or diminish the action of the manures.*

(1) By the botanical composition of the herbage of the plots (before the manures are added) in relation to the type of grass land to which they belong; that is to say, by the position of each of the fundamental species in relation to its cardinal figures.

(2) By the prevailing meteorological conditions. Exceptional

seasons[1] may upset the equilibrium between environment and community sufficiently to force the distribution of some species outside their cardinal figures and so occasion type alteration.

Many species are, moreover, very sensitive to the available water of the habitat and will fluctuate within the limits of their cardinal figures under relatively slight seasonal change. Such in particular are Yorkshire fog, upright brome and the meadow grasses.

B. *Factors which are indirectly dependent on the nature of the manures added.*

(1) By the *rôle* the manure exercises in merely disturbing the prevailing equilibrium. This re-awakens competitive interaction, since the altered conditions will tend to favour the growth of some species more than of others, and so initiate a quantitative readjustment between the contributing species.

Silica sand and other non-nutritious dressings are competent to act in this manner.

(2) By the effect the treatment may have on the physical, chemical, and biological properties of the soil, and in proportion as this modifies the texture of the soil, its capacity for holding water and the availability of plant foods. Farmyard manure (and other organic manures) and lime or limestone (in its various forms) are most competent to act in this manner. The actions of basic slag and common salt, and, to a less extent, also those of superphosphate, kainit, and ammonium sulphate (when continually added) are partially of a soil-controlling nature. The soil-controlling manures are more competent than any others to occasion "type" alterations. They are capable of completely altering the physiognomy of grass land of a strongly indigenous character; probably for the simple reason that such "types" are invariably associated with an equally characteristic soil. Good examples are (*a*) grassland dominated by the upright brome on the calcareous soils of the Cotswolds [(2)], here farmyard manure or guano continually added have entirely altered the character of the association, for the brome has fallen far below its minimum, whilst rye grass, golden oat and meadow foxtail have all exceeded their maxima; (*b*) grassland dominated by bent at Garforth [(10)], and near Lancaster [(11)], in the former case farmyard manure has decreased the bent by over 50 % and greatly added to meadow foxtail, cocksfoot and weeds; in the latter case great reduction of bent has been effected by ground limestone.

[1] *e.g.* 1911, see (9) and (4).

C. *Factors which are dependent on the chemical composition of the manures added.*

(1) By (*a*) the individual appetites of the several species for the particular plant-foods in the form they are added, and (*b*) the physiologically depressing effect particular manures may produce on individual species. These direct chemical influences are very difficult to estimate, since they tend more than any others to be masked by all the factors under discussion. The relation of the species to their cardinal figures is important in this connection. It would seem that such as stand near their optimum figure (in reality a figure rather below the optimum is better) are in a position to best show their chemical affinities with the manures applied, since they are then capable of wide fluctuations within the limits of their cardinal figures. When species stand near their minima their behaviour is irregular towards all manures except those for which they have a very marked avidity[1]. When near their maxima they are seldom increased (except by the soil-controlling manures), and are then sometimes decreased by manures which would otherwise increase them. If the action of manures on species are judged by these standards certain marked avidities and the reverse are apparent.

Perennial rye grass and cocksfoot have strong avidities for inorganic nitrogen, bent has a considerable avidity for ammonium sulphate, erect brome has an avidity for potash salts, which may even carry it beyond its maximum figure. Rough stalked meadow grass and smooth stalked meadow grass have very strong avidities for nitrate and ammonium salts respectively. The remarkable avidity of the leguminosae for phosphatic manures, especially of Dutch clover for basic slag, is somewhat exceptional. The behaviour of Dutch clover under slag bears no relation to its cardinal figures; it may be increased from an insignificant position to far above its maximum figure. This suggests a twofold action on the part of basic slag (on those soils where it is especially successful) in the first place the clover responds on account of a marked avidity for phosphates, in the second place the slag, in its capacity as a soil-controller, modifies the habitat in a direction even more favourable to this plant. Reductions of species under manures are more often to be explained by indirect than direct influences. It has been shown that reduction of perennial rye grass under phosphates

[1] Or towards those which have a marked depressing influence on such species.

is directly due to interspecific competition. Large reductions of bent under basic slag (which are not universal) are almost certainly due to the combined influences of interspecific competition and soil control.

Inorganic nitrogen, however, would seem to have a depressing influence on clovers (which may be reduced even when standing at their minima).

The most striking effect is that of ammonium sulphate (especially with full mineral dressings) on certain weeds (*e.g.* ribgrass, buttercups, daisies, hawkweeds, etc.) which it reduces even when near their minima.

The final re-adjustment will, of course, be regulated by the interaction of all the separate factors which are operative.

On the application of the dressing, the comparatively few species, which may have a marked avidity, or the reverse, for the substances added, will rapidly increase or decrease, as the case may be. Interspecific competition is thus re-awakened, with the result that the far larger number of species without any marked partiality towards the substances added will readjust themselves in relation to their cardinal figures, rather than in relation to the altered availability of any particular plant food or plant foods.

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